

CONFIDENTIAL

Analog data to address EPA's ecotoxicity concerns for P-17-0119

Introduction:

In order to address EPA's concerns for the aquatic toxicity of P-17-0119, we are providing the following test data conducted on a close analog of the PMN substance:

- 1) Acute Toxicity to *Daphnia magna*
- 2) Algal growth inhibition assay

Representative Chemical Structure of the analog:

Monomers and reactants used to manufacture the analog polymer:

CAS RN and CAS Name – CAS RN:

Synonyms:

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GPC Data:

Mn: 11,122 D

Wt. % < 1000: 1.31 %

Wt. % < 500: 0.00%

Summary of Aquatic Toxicity Tests:

| Study Title | Result |
|---------------------------------|---|
| Acute Toxicity to Daphnia magna | 48 hour EC ₅₀ value: >10g/L |
| Algal growth inhibition study | E ₀ L ₅₀ (72 h): >10g/L |



ClientService

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CAS CLIENT SERVICES

Order Number

Customer's

Substance ID

Processing Result

Comments

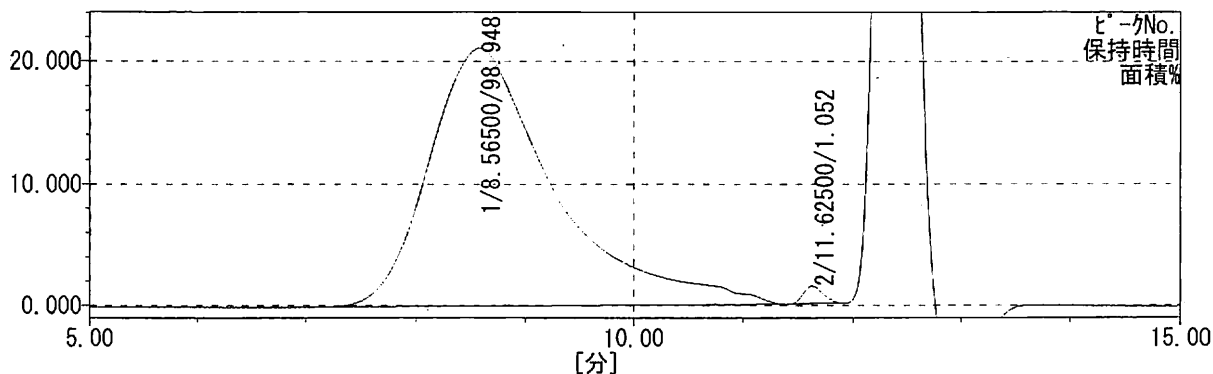
タイトル:

<表題部>

Sample Name サンプル名 :
 データベース名 : 201003.mdb
 保存データベース名 : 201003100001
 マットデータベース名 : 201003100001
 収集属性 : 独立
 計算Ch : Ch 1

測定日時 : 2009/09/29 15:44:30
 計算日時 : 2010/03/23 14:47:47
 シリアル番号 : 10
 カップ番号 : 2
 収集時間[分] : 5.00 - 15.00
 計算方法 : 分子量計算

[mV]



<Ch1 分子量計算結果>

ピーク 1 ベースピーク

| | [分] | [mV] | [MOL] |
|-----------|-------|--------|---------|
| ピークスタート : | 7.11 | -0.130 | 419,537 |
| ピークトップ : | 8.56 | 21.076 | 49,816 |
| ピークエント : | 11.35 | 0.114 | 582 |

面積[mV・秒] : 1,684.058
 高さ比[%] : 93.571
 高さ[mV] : 21.122
 [η] : 49,378.56167

| | |
|---------|-----------|
| Mn | : 11.122 |
| Mw | : 49.379 |
| Mz | : 91.386 |
| Mz+1 | : 129.456 |
| Mv | : 49.379 |
| Mp | : 51.206 |
| Mz/Mw | : 1.851 |
| Mw/Mn | : 4.440 |
| Mz+1/Mw | : 2.622 |

ピーク 2 ベースピーク

| | [分] | [mV] | [MOL] |
|-----------|-------|-------|-------|
| ピークスタート : | 11.44 | 0.130 | 549 |
| ピークトップ : | 11.63 | 1.612 | 497 |
| ピークエント : | 11.88 | 0.202 | 465 |

面積[mV・秒] : 17.902
 高さ比[%] : 6.429
 高さ[mV] : 1.451
 [η] : 495.45749

| | |
|---------|---------|
| Mn | : 495 |
| Mw | : 495 |
| Mz | : 496 |
| Mz+1 | : 496 |
| Mv | : 495 |
| Mp | : 490 |
| Mz/Mw | : 1.001 |
| Mw/Mn | : 1.001 |
| Mz+1/Mw | : 1.002 |

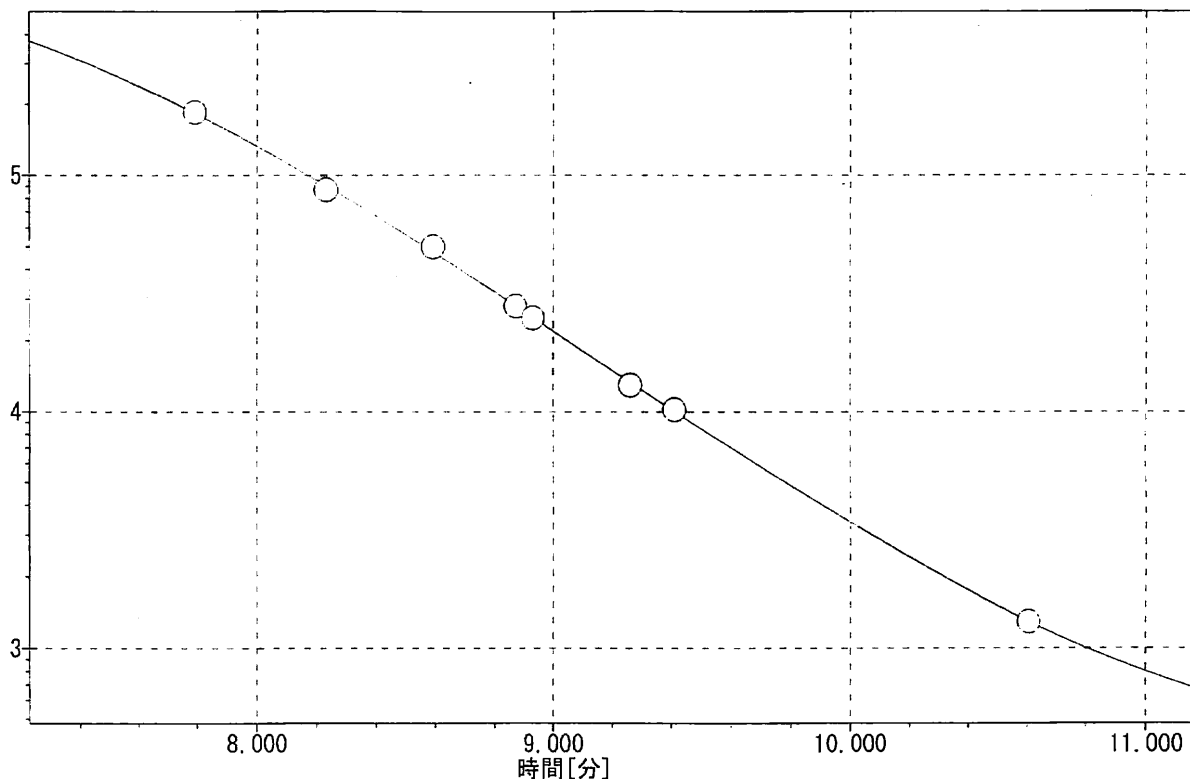
<分析条件> <GPC Analysis condition>

Column カラム : TSKgel SuperH2M-M X2
 Sol. No. カラムロットNo : K0022, K0023
 Flow rate 流量 : 0.35 ml/min
 Operator 測定者 :
 Detector 検出器 : RI-022V
 Content 濃度 : 6 mg/ml
 Injection 注入量 : 3 μl
 Column Temp. カラム温度 : 40 °C
 Dilute 溶離液 : THF

データ名 : 201003.mdb
 保存データ名 : 201003100001
 マットデータ名 : 201003100001
 Ch番号 : 1
 検量線式 : 3次 : At^3+Bt^2+Ct+D
 補正 : 補正なし

Caribration of GPC

[logM]



| <データ> 時間[分] Retention time | 分子量 Molecular | 誤差[%] | 重み | 作成データ名 |
|----------------------------------|------------------|----------|----|--------|
| 7.79 | 185000 | 0.91106 | 1 | |
| 8.23 | 87000 | -4.00228 | 1 | |
| 8.59 | 50000 | 4.84774 | 1 | |
| 8.87 | 28000 | -0.52567 | 1 | |
| 8.93 | 25000 | -0.44226 | 1 | |
| 9.26 | 13000 | -2.71655 | 1 | |
| 9.41 | 10200 | 1.64717 | 1 | |
| 10.61 | 1300 | 0.02484 | 1 | |

式係数

A= 3.504818E-002
 B= -9.607648E-001
 C= 7.946774E+000
 D= -1.490732E+001

相関係数: -1.000

↑
Polystylen standard sample

データ名 : 201003.mdb 微分曲線換算係数 : Ch.1 1.00 Ch.2 1.00
保存データ名 : 201003100001 微分・積分計算式 : 標準
ワットデータ名 : 201003100001 サンプリングレイト[msec] : 300

チャネル: Ch1

ピークNo.: 1

時間[分] : 7.11 ~ 8.56 ~ 11.35

Molecular

Integral distribution

| 時間[分] | 分子量 | 微分分布[面積] | 微分分布[高さ] | 積分分布 |
|-------|---------|----------|----------|----------|
| 7.11 | 419.537 | 0.0000 | 0.0000 | 100.0000 |
| 7.12 | 417.604 | 0.0203 | 5.3865 | 100.0000 |
| 7.12 | 415.670 | 0.0051 | 1.3478 | 99.9999 |
| 7.13 | 413.735 | 0.0076 | 2.0112 | 99.9999 |
| 7.13 | 411.798 | 0.0248 | 6.6037 | 99.9999 |
| 7.14 | 409.861 | 0.0222 | 5.9063 | 99.9998 |
| 7.14 | 407.923 | 0.0369 | 9.8045 | 99.9998 |
| 7.15 | 405.985 | 0.0342 | 9.0981 | 99.9997 |
| 7.15 | 404.045 | 0.0145 | 3.8567 | 99.9997 |
| 7.16 | 402.106 | 0.0390 | 10.3718 | 99.9996 |
| 7.16 | 400.166 | 0.0243 | 6.4702 | 99.9995 |
| 7.17 | 398.226 | 0.0239 | 6.3435 | 99.9995 |
| 7.17 | 396.286 | 0.0213 | 5.6719 | 99.9994 |
| 7.18 | 394.346 | 0.0022 | 0.5749 | 99.9994 |
| 7.18 | 392.406 | 0.0164 | 4.3482 | 99.9994 |
| 7.19 | 390.467 | 0.0965 | 25.6442 | 99.9992 |
| 7.19 | 388.527 | 0.0608 | 16.1566 | 99.9991 |
| 7.20 | 386.589 | 0.0073 | 1.9383 | 99.9990 |
| 7.20 | 384.651 | 0.0555 | 14.7604 | 99.9989 |
| 7.21 | 382.713 | 0.1340 | 35.6126 | 99.9986 |
| 7.21 | 380.777 | 0.1311 | 34.8320 | 99.9983 |
| 7.22 | 378.842 | 0.0960 | 25.5215 | 99.9981 |
| 7.22 | 376.907 | 0.0933 | 24.7946 | 99.9979 |
| 7.23 | 374.974 | 0.1224 | 32.5349 | 99.9976 |
| 7.23 | 373.042 | 0.1196 | 31.7838 | 99.9974 |
| 7.24 | 371.111 | 0.1641 | 43.6176 | 99.9970 |
| 7.24 | 369.182 | 0.1611 | 42.8250 | 99.9966 |
| 7.25 | 367.255 | 0.2833 | 75.2875 | 99.9960 |
| 7.25 | 365.329 | 0.4044 | 107.4685 | 99.9951 |
| 7.26 | 363.405 | 0.3539 | 94.0521 | 99.9942 |
| 7.26 | 361.483 | 0.3038 | 80.7519 | 99.9935 |
| 7.27 | 359.563 | 0.2696 | 71.6508 | 99.9929 |
| 7.27 | 357.645 | 0.2969 | 78.8985 | 99.9922 |
| 7.28 | 355.729 | 0.2630 | 69.8834 | 99.9916 |
| 7.28 | 353.816 | 0.2597 | 69.0120 | 99.9910 |
| 7.29 | 351.904 | 0.2867 | 76.1827 | 99.9903 |
| 7.29 | 349.996 | 0.4188 | 111.2994 | 99.9893 |
| 7.30 | 348.090 | 0.5199 | 138.1617 | 99.9881 |
| 7.30 | 346.186 | 0.5455 | 144.9669 | 99.9868 |
| 7.31 | 344.285 | 0.5411 | 143.8146 | 99.9855 |
| 7.31 | 342.388 | 0.5072 | 134.8004 | 99.9843 |
| 7.32 | 340.493 | 0.5031 | 133.7008 | 99.9831 |
| 7.32 | 338.601 | 0.4843 | 128.7061 | 99.9819 |
| 7.33 | 336.712 | 0.5242 | 139.3117 | 99.9806 |
| 7.33 | 334.826 | 0.6367 | 169.2098 | 99.9791 |
| 7.34 | 332.944 | 0.7483 | 198.8780 | 99.9772 |
| 7.34 | 331.065 | 0.8302 | 220.6302 | 99.9752 |
| 7.35 | 329.189 | 0.8826 | 234.5579 | 99.9730 |
| 7.35 | 327.317 | 0.8772 | 233.1194 | 99.9708 |
| 7.36 | 325.449 | 0.8432 | 224.0916 | 99.9687 |
| 7.36 | 323.584 | 0.8380 | 222.7079 | 99.9666 |
| 7.37 | 321.723 | 0.8754 | 232.6561 | 99.9644 |
| 7.37 | 319.866 | 0.9409 | 250.0513 | 99.9621 |
| 7.38 | 318.013 | 1.0200 | 271.0665 | 99.9595 |
| 7.38 | 316.164 | 1.1546 | 306.8576 | 99.9566 |
| 7.39 | 314.318 | 1.2743 | 338.6741 | 99.9533 |
| 7.39 | 312.477 | 1.3653 | 362.8551 | 99.9498 |
| 7.40 | 310.641 | 1.3168 | 349.9517 | 99.9464 |
| 7.40 | 308.808 | 1.3378 | 355.5356 | 99.9430 |
| 7.41 | 306.980 | 1.3725 | 364.7491 | 99.9395 |

| | | | | |
|------|---------|---------|-----------|---------|
| 7.41 | 305.156 | 1.4344 | 381.2065 | 99.9357 |
| 7.42 | 303.337 | 1.5096 | 401.1926 | 99.9318 |
| 7.42 | 301.522 | 1.6252 | 431.9257 | 99.9276 |
| 7.43 | 299.712 | 1.8217 | 484.1394 | 99.9228 |
| 7.43 | 297.907 | 1.9355 | 514.3863 | 99.9177 |
| 7.44 | 296.106 | 2.0756 | 551.6126 | 99.9122 |
| 7.44 | 294.310 | 2.0666 | 549.2348 | 99.9068 |
| 7.45 | 292.519 | 2.0980 | 557.5764 | 99.9012 |
| 7.45 | 290.733 | 2.1158 | 562.3137 | 99.8956 |
| 7.46 | 288.952 | 2.1202 | 563.4828 | 99.8899 |
| 7.46 | 287.176 | 2.2176 | 589.3641 | 99.8839 |
| 7.47 | 285.406 | 2.3938 | 636.1958 | 99.8775 |
| 7.47 | 283.640 | 2.5821 | 686.2363 | 99.8705 |
| 7.48 | 281.880 | 2.7298 | 725.4706 | 99.8631 |
| 7.48 | 280.124 | 2.8765 | 764.4595 | 99.8553 |
| 7.49 | 278.375 | 2.9438 | 782.3629 | 99.8473 |
| 7.49 | 276.630 | 2.9326 | 779.3827 | 99.8393 |
| 7.50 | 274.891 | 3.0254 | 804.0446 | 99.8310 |
| 7.50 | 273.158 | 3.1177 | 828.5594 | 99.8224 |
| 7.51 | 271.430 | 3.2481 | 863.2205 | 99.8134 |
| 7.51 | 269.708 | 3.4292 | 911.3543 | 99.8039 |
| 7.52 | 267.991 | 3.6477 | 969.4280 | 99.7938 |
| 7.52 | 266.280 | 3.8138 | 1013.5597 | 99.7832 |
| 7.53 | 264.575 | 3.9788 | 1057.4324 | 99.7721 |
| 7.53 | 262.876 | 4.1048 | 1090.9145 | 99.7606 |
| 7.54 | 261.182 | 4.1541 | 1103.9964 | 99.7489 |
| 7.54 | 259.494 | 4.2157 | 1120.3685 | 99.7370 |
| 7.55 | 257.812 | 4.3147 | 1146.6983 | 99.7248 |
| 7.55 | 256.137 | 4.5137 | 1199.5899 | 99.7120 |
| 7.56 | 254.467 | 4.7743 | 1268.8249 | 99.6984 |
| 7.56 | 252.803 | 4.9709 | 1321.0736 | 99.6842 |
| 7.57 | 251.145 | 5.1539 | 1369.7222 | 99.6695 |
| 7.57 | 249.494 | 5.3484 | 1421.4028 | 99.6541 |
| 7.58 | 247.848 | 5.6037 | 1489.2519 | 99.6380 |
| 7.58 | 246.209 | 5.6971 | 1514.0760 | 99.6216 |
| 7.59 | 244.576 | 5.8146 | 1545.3168 | 99.6047 |
| 7.59 | 242.949 | 5.9316 | 1576.3958 | 99.5875 |
| 7.60 | 241.329 | 6.2193 | 1652.8626 | 99.5694 |
| 7.60 | 239.715 | 6.4689 | 1719.1863 | 99.5505 |
| 7.61 | 238.107 | 6.7171 | 1785.1591 | 99.5309 |
| 7.61 | 236.506 | 6.9033 | 1834.6529 | 99.5106 |
| 7.62 | 234.911 | 7.1249 | 1893.5462 | 99.4897 |
| 7.62 | 233.323 | 7.3937 | 1964.9730 | 99.4678 |
| 7.63 | 231.741 | 7.5647 | 2010.4288 | 99.4455 |
| 7.63 | 230.165 | 7.6869 | 2042.8915 | 99.4227 |
| 7.64 | 228.596 | 7.9282 | 2107.0326 | 99.3991 |
| 7.64 | 227.034 | 8.2400 | 2189.9020 | 99.3745 |
| 7.65 | 225.478 | 8.4907 | 2256.5214 | 99.3491 |
| 7.65 | 223.929 | 8.7877 | 2335.4450 | 99.3228 |
| 7.66 | 222.386 | 9.0595 | 2407.6783 | 99.2955 |
| 7.66 | 220.851 | 9.3654 | 2488.9847 | 99.2673 |
| 7.67 | 219.321 | 9.6581 | 2566.7609 | 99.2381 |
| 7.67 | 217.799 | 9.8787 | 2625.4063 | 99.2082 |
| 7.68 | 216.283 | 10.0632 | 2674.4226 | 99.1776 |
| 7.68 | 214.774 | 10.3755 | 2757.4256 | 99.1461 |
| 7.69 | 213.272 | 10.7213 | 2849.3426 | 99.1133 |
| 7.69 | 211.777 | 11.0423 | 2934.6360 | 99.0796 |
| 7.70 | 210.288 | 11.3850 | 3025.7053 | 99.0446 |
| 7.70 | 208.806 | 11.7144 | 3113.2718 | 99.0086 |
| 7.71 | 207.331 | 12.0309 | 3197.3637 | 98.9715 |
| 7.71 | 205.863 | 12.3458 | 3281.0729 | 98.9334 |
| 7.72 | 204.402 | 12.6709 | 3367.4612 | 98.8941 |
| 7.72 | 202.947 | 12.9945 | 3453.4618 | 98.8538 |
| 7.73 | 201.500 | 13.3167 | 3539.0798 | 98.8123 |
| 7.73 | 200.059 | 13.6716 | 3633.4251 | 98.7697 |
| 7.74 | 198.626 | 14.0251 | 3727.3544 | 98.7258 |
| 7.74 | 197.199 | 14.4338 | 3835.9768 | 98.6806 |
| 7.75 | 195.779 | 14.8067 | 3935.0822 | 98.6341 |
| 7.75 | 194.366 | 15.1554 | 4027.7463 | 98.5863 |
| 7.76 | 192.960 | 15.5139 | 4123.0153 | 98.5374 |

| | | | | |
|-------|----------|----------|-------------|----------|
| 7. 76 | 191, 561 | 15. 9046 | 4226. 8596 | 98. 4870 |
| 7. 77 | 190, 169 | 16. 2824 | 4327. 2785 | 98. 4354 |
| 7. 77 | 188, 784 | 16. 6587 | 4427. 2771 | 98. 3825 |
| 7. 78 | 187, 406 | 17. 0558 | 4532. 8061 | 98. 3281 |
| 7. 78 | 186, 035 | 17. 4735 | 4643. 8307 | 98. 2724 |
| 7. 79 | 184, 671 | 17. 9341 | 4766. 2355 | 98. 2150 |
| 7. 79 | 183, 314 | 18. 3817 | 4885. 1837 | 98. 1560 |
| 7. 80 | 181, 964 | 18. 7942 | 4994. 8086 | 98. 0956 |
| 7. 80 | 180, 621 | 19. 2050 | 5103. 9938 | 98. 0338 |
| 7. 81 | 179, 285 | 19. 7136 | 5239. 1458 | 97. 9701 |
| 7. 81 | 177, 956 | 20. 2091 | 5370. 8280 | 97. 9048 |
| 7. 82 | 176, 634 | 20. 6256 | 5481. 5409 | 97. 8379 |
| 7. 82 | 175, 319 | 21. 0845 | 5603. 4827 | 97. 7694 |
| 7. 83 | 174, 011 | 21. 5525 | 5727. 8591 | 97. 6992 |
| 7. 83 | 172, 710 | 22. 0077 | 5848. 8534 | 97. 6274 |
| 7. 84 | 171, 416 | 22. 4940 | 5978. 0743 | 97. 5539 |
| 7. 84 | 170, 129 | 22. 9892 | 6109. 6932 | 97. 4786 |
| 7. 85 | 168, 849 | 23. 4826 | 6240. 8140 | 97. 4015 |
| 7. 85 | 167, 576 | 24. 0283 | 6385. 8339 | 97. 3224 |
| 7. 86 | 166, 310 | 24. 6151 | 6541. 7982 | 97. 2413 |
| 7. 86 | 165, 052 | 25. 0811 | 6665. 6472 | 97. 1584 |
| 7. 87 | 163, 800 | 25. 5670 | 6794. 7678 | 97. 0738 |
| 7. 87 | 162, 555 | 26. 0833 | 6931. 9859 | 96. 9873 |
| 7. 88 | 161, 317 | 26. 5870 | 7065. 8594 | 96. 8989 |
| 7. 88 | 160, 086 | 27. 1104 | 7204. 9419 | 96. 8087 |
| 7. 89 | 158, 862 | 27. 6639 | 7352. 0436 | 96. 7164 |
| 7. 89 | 157, 645 | 28. 2047 | 7495. 7914 | 96. 6221 |
| 7. 90 | 156, 435 | 28. 8288 | 7661. 6490 | 96. 5255 |
| 7. 90 | 155, 232 | 29. 4719 | 7832. 5634 | 96. 4266 |
| 7. 91 | 154, 036 | 30. 0068 | 7974. 7197 | 96. 3257 |
| 7. 91 | 152, 846 | 30. 5611 | 8122. 0122 | 96. 2227 |
| 7. 92 | 151, 664 | 31. 1239 | 8271. 6097 | 96. 1177 |
| 7. 92 | 150, 489 | 31. 6744 | 8417. 9032 | 96. 0105 |
| 7. 93 | 149, 320 | 32. 2546 | 8572. 0927 | 95. 9013 |
| 7. 93 | 148, 159 | 32. 8433 | 8728. 5586 | 95. 7898 |
| 7. 94 | 147, 004 | 33. 4406 | 8887. 2919 | 95. 6760 |
| 7. 94 | 145, 856 | 34. 1091 | 9064. 9567 | 95. 5598 |
| 7. 95 | 144, 715 | 34. 7963 | 9247. 5835 | 95. 4410 |
| 7. 95 | 143, 581 | 35. 4291 | 9415. 7684 | 95. 3199 |
| 7. 96 | 142, 454 | 36. 0183 | 9572. 3580 | 95. 1964 |
| 7. 96 | 141, 333 | 36. 5849 | 9722. 9365 | 95. 0709 |
| 7. 97 | 140, 220 | 37. 2016 | 9886. 8155 | 94. 9430 |
| 7. 97 | 139, 113 | 37. 8370 | 10055. 6809 | 94. 8126 |
| 7. 98 | 138, 013 | 38. 4807 | 10226. 7660 | 94. 6798 |
| 7. 98 | 136, 920 | 39. 1534 | 10405. 5412 | 94. 5445 |
| 7. 99 | 135, 833 | 39. 8652 | 10594. 7039 | 94. 4064 |
| 7. 99 | 134, 754 | 40. 5953 | 10788. 7450 | 94. 2656 |
| 8. 00 | 133, 681 | 41. 2514 | 10963. 1208 | 94. 1222 |
| 8. 00 | 132, 614 | 41. 8954 | 11134. 2576 | 93. 9764 |
| 8. 01 | 131, 555 | 42. 5068 | 11296. 7450 | 93. 8282 |
| 8. 01 | 130, 502 | 43. 1878 | 11477. 7390 | 93. 6773 |
| 8. 02 | 129, 455 | 43. 8261 | 11647. 3811 | 93. 5240 |
| 8. 02 | 128, 416 | 44. 4931 | 11824. 6442 | 93. 3680 |
| 8. 03 | 127, 383 | 45. 2292 | 12020. 2826 | 93. 2093 |
| 8. 03 | 126, 356 | 46. 0139 | 12228. 8233 | 93. 0475 |
| 8. 04 | 125, 336 | 46. 7357 | 12420. 6364 | 92. 8828 |
| 8. 04 | 124, 323 | 47. 4756 | 12617. 2795 | 92. 7153 |
| 8. 05 | 123, 317 | 48. 0824 | 12778. 5414 | 92. 5454 |
| 8. 05 | 122, 316 | 48. 7681 | 12960. 7757 | 92. 3728 |
| 8. 06 | 121, 323 | 49. 4620 | 13145. 1891 | 92. 1975 |
| 8. 06 | 120, 336 | 50. 1440 | 13326. 4422 | 92. 0194 |
| 8. 07 | 119, 355 | 50. 8442 | 13512. 5409 | 91. 8386 |
| 8. 07 | 118, 381 | 51. 6426 | 13724. 7311 | 91. 6546 |
| 8. 08 | 117, 413 | 52. 3990 | 13925. 7392 | 91. 4677 |
| 8. 08 | 116, 452 | 53. 1434 | 14123. 5714 | 91. 2778 |
| 8. 09 | 115, 497 | 53. 8759 | 14318. 2471 | 91. 0849 |
| 8. 09 | 114, 548 | 54. 5668 | 14501. 8603 | 90. 8893 |
| 8. 10 | 113, 606 | 55. 1964 | 14669. 1910 | 90. 6912 |
| 8. 10 | 112, 670 | 55. 8642 | 14846. 6529 | 90. 4903 |
| 8. 11 | 111, 740 | 56. 5204 | 15021. 0560 | 90. 2868 |

| | | | | |
|-------|----------|----------|-------------|----------|
| 8. 11 | 110, 817 | 57. 2837 | 15223. 9253 | 90. 0802 |
| 8. 12 | 109, 900 | 58. 0452 | 15426. 2986 | 89. 8706 |
| 8. 12 | 108, 989 | 58. 8048 | 15628. 1824 | 89. 6579 |
| 8. 13 | 108, 084 | 59. 5233 | 15819. 1274 | 89. 4423 |
| 8. 13 | 107, 186 | 60. 2205 | 16004. 4065 | 89. 2238 |
| 8. 14 | 106, 294 | 60. 9455 | 16197. 0801 | 89. 0024 |
| 8. 14 | 105, 408 | 61. 5513 | 16358. 0816 | 88. 7785 |
| 8. 15 | 104, 528 | 62. 1753 | 16523. 9353 | 88. 5519 |
| 8. 15 | 103, 654 | 62. 8371 | 16699. 8142 | 88. 3227 |
| 8. 16 | 102, 786 | 63. 6047 | 16903. 8193 | 88. 0903 |
| 8. 16 | 101, 924 | 64. 3414 | 17099. 6064 | 87. 8549 |
| 8. 17 | 101, 068 | 65. 0863 | 17297. 5512 | 87. 6164 |
| 8. 17 | 100, 219 | 65. 7518 | 17474. 4235 | 87. 3751 |
| 8. 18 | 99, 375 | 66. 4644 | 17663. 8162 | 87. 1309 |
| 8. 18 | 98, 537 | 67. 1272 | 17839. 9467 | 86. 8839 |
| 8. 19 | 97, 705 | 67. 7305 | 18000. 3033 | 86. 6344 |
| 8. 19 | 96, 879 | 68. 3134 | 18155. 2199 | 86. 3823 |
| 8. 20 | 96, 059 | 68. 9531 | 18325. 2261 | 86. 1276 |
| 8. 20 | 95, 245 | 69. 6494 | 18510. 2648 | 85. 8700 |
| 8. 21 | 94, 437 | 70. 3058 | 18684. 7175 | 85. 6095 |
| 8. 21 | 93, 634 | 70. 9610 | 18858. 8414 | 85. 3463 |
| 8. 22 | 92, 837 | 71. 6341 | 19037. 7406 | 85. 0803 |
| 8. 22 | 92, 046 | 72. 2581 | 19203. 5767 | 84. 8116 |
| 8. 23 | 91, 261 | 72. 8619 | 19364. 0329 | 84. 5403 |
| 8. 23 | 90, 481 | 73. 4168 | 19511. 5145 | 84. 2666 |
| 8. 24 | 89, 708 | 73. 9709 | 19658. 7564 | 83. 9904 |
| 8. 24 | 88, 939 | 74. 5621 | 19815. 8956 | 83. 7117 |
| 8. 25 | 88, 177 | 75. 1620 | 19975. 3077 | 83. 4304 |
| 8. 25 | 87, 420 | 75. 7418 | 20129. 4069 | 83. 1466 |
| 8. 26 | 86, 668 | 76. 3397 | 20288. 3114 | 82. 8602 |
| 8. 26 | 85, 922 | 76. 9272 | 20444. 4446 | 82. 5712 |
| 8. 27 | 85, 182 | 77. 5043 | 20597. 8202 | 82. 2797 |
| 8. 27 | 84, 447 | 78. 0238 | 20735. 8790 | 81. 9859 |
| 8. 28 | 83, 717 | 78. 5048 | 20863. 7027 | 81. 6899 |
| 8. 28 | 82, 993 | 78. 9663 | 20986. 3477 | 81. 3918 |
| 8. 29 | 82, 274 | 79. 5120 | 21131. 3950 | 81. 0913 |
| 8. 29 | 81, 561 | 80. 0288 | 21268. 7380 | 80. 7885 |
| 8. 30 | 80, 853 | 80. 5355 | 21403. 4060 | 80. 4834 |
| 8. 30 | 80, 151 | 81. 0604 | 21542. 9010 | 80. 1760 |
| 8. 31 | 79, 453 | 81. 5377 | 21669. 7529 | 79. 8664 |
| 8. 31 | 78, 761 | 82. 0239 | 21798. 9533 | 79. 5546 |
| 8. 32 | 78, 075 | 82. 5001 | 21925. 5209 | 79. 2407 |
| 8. 32 | 77, 393 | 82. 9478 | 22044. 4967 | 78. 9246 |
| 8. 33 | 76, 717 | 83. 3296 | 22145. 9792 | 78. 6068 |
| 8. 33 | 76, 045 | 83. 7859 | 22267. 2245 | 78. 2869 |
| 8. 34 | 75, 379 | 84. 2416 | 22388. 3536 | 77. 9648 |
| 8. 34 | 74, 718 | 84. 6784 | 22504. 4210 | 77. 6408 |
| 8. 35 | 74, 063 | 85. 0682 | 22608. 0312 | 77. 3148 |
| 8. 35 | 73, 412 | 85. 4857 | 22718. 9777 | 76. 9870 |
| 8. 36 | 72, 766 | 85. 8843 | 22824. 9081 | 76. 6572 |
| 8. 36 | 72, 125 | 86. 3011 | 22935. 6949 | 76. 3255 |
| 8. 37 | 71, 489 | 86. 6714 | 23034. 0983 | 75. 9921 |
| 8. 37 | 70, 858 | 87. 0230 | 23127. 5347 | 75. 6569 |
| 8. 38 | 70, 232 | 87. 3652 | 23218. 4774 | 75. 3200 |
| 8. 38 | 69, 611 | 87. 7534 | 23321. 6555 | 74. 9813 |
| 8. 39 | 68, 995 | 88. 1046 | 23414. 9767 | 74. 6409 |
| 8. 39 | 68, 384 | 88. 4648 | 23510. 7143 | 74. 2988 |
| 8. 40 | 67, 777 | 88. 7881 | 23596. 6342 | 73. 9550 |
| 8. 40 | 67, 175 | 89. 1205 | 23684. 9844 | 73. 6097 |
| 8. 41 | 66, 578 | 89. 4345 | 23768. 4366 | 73. 2627 |
| 8. 41 | 65, 986 | 89. 7394 | 23849. 4458 | 72. 9143 |
| 8. 42 | 65, 398 | 90. 0442 | 23930. 4569 | 72. 5643 |
| 8. 42 | 64, 815 | 90. 3399 | 24009. 0377 | 72. 2128 |
| 8. 43 | 64, 237 | 90. 6264 | 24085. 1971 | 71. 8599 |
| 8. 43 | 63, 663 | 90. 8948 | 24156. 5144 | 71. 5055 |
| 8. 44 | 63, 094 | 91. 1632 | 24227. 8585 | 71. 1498 |
| 8. 44 | 62, 529 | 91. 4683 | 24308. 9284 | 70. 7926 |
| 8. 45 | 61, 969 | 91. 6913 | 24368. 2104 | 70. 4341 |
| 8. 45 | 61, 414 | 91. 8873 | 24420. 2860 | 70. 0746 |
| 8. 46 | 60, 863 | 92. 1472 | 24489. 3531 | 69. 7137 |

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| 8. 46 | 60, 316 | 92. 4072 | 24558. 4579 | 69. 3515 |
| 8. 47 | 59, 774 | 92. 5947 | 24608. 2956 | 68. 9882 |
| 8. 47 | 59, 236 | 92. 7734 | 24655. 7971 | 68. 6238 |
| 8. 48 | 58, 703 | 92. 9253 | 24696. 1517 | 68. 2586 |
| 8. 48 | 58, 173 | 93. 1228 | 24748. 6341 | 67. 8922 |
| 8. 49 | 57, 649 | 93. 2934 | 24793. 9754 | 67. 5249 |
| 8. 49 | 57, 128 | 93. 4282 | 24829. 7925 | 67. 1567 |
| 8. 50 | 56, 612 | 93. 5994 | 24875. 3156 | 66. 7875 |
| 8. 50 | 56, 100 | 93. 7801 | 24923. 3249 | 66. 4173 |
| 8. 51 | 55, 592 | 93. 9611 | 24971. 4186 | 66. 0461 |
| 8. 51 | 55, 088 | 94. 0162 | 24986. 0742 | 65. 6743 |
| 8. 52 | 54, 589 | 94. 0719 | 25000. 8714 | 65. 3020 |
| 8. 52 | 54, 093 | 94. 1371 | 25018. 2006 | 64. 9291 |
| 8. 53 | 53, 602 | 94. 1938 | 25033. 2786 | 64. 5557 |
| 8. 53 | 53, 115 | 94. 2421 | 25046. 1114 | 64. 1819 |
| 8. 54 | 52, 632 | 94. 2640 | 25051. 9351 | 63. 8076 |
| 8. 54 | 52, 152 | 94. 2955 | 25060. 2980 | 63. 4329 |
| 8. 55 | 51, 677 | 94. 3902 | 25085. 4804 | 63. 0575 |
| 8. 55 | 51, 206 | 94. 4586 | 25103. 6516 | 62. 6816 |
| 8. 56 | 50, 739 | 94. 4112 | 25091. 0554 | 62. 3055 |
| 8. 56 | 50, 275 | 94. 3466 | 25073. 8930 | 61. 9295 |
| 8. 57 | 49, 816 | 94. 3274 | 25068. 7904 | 61. 5532 |
| 8. 57 | 49, 360 | 94. 2286 | 25042. 5134 | 61. 1770 |
| 8. 58 | 48, 908 | 94. 1305 | 25016. 4405 | 60. 8010 |
| 8. 58 | 48, 460 | 94. 0509 | 24995. 3074 | 60. 4250 |
| 8. 59 | 48, 016 | 94. 0078 | 24983. 8361 | 60. 0489 |
| 8. 59 | 47, 576 | 93. 9742 | 24974. 9105 | 59. 6726 |
| 8. 60 | 47, 139 | 93. 9057 | 24956. 7085 | 59. 2964 |
| 8. 60 | 46, 706 | 93. 7757 | 24922. 1649 | 58. 9204 |
| 8. 61 | 46, 276 | 93. 5755 | 24868. 9538 | 58. 5449 |
| 8. 61 | 45, 851 | 93. 3939 | 24820. 6962 | 58. 1700 |
| 8. 62 | 45, 429 | 93. 1866 | 24765. 5977 | 57. 7956 |
| 8. 62 | 45, 010 | 92. 9979 | 24715. 4465 | 57. 4217 |
| 8. 63 | 44, 595 | 92. 7923 | 24660. 8177 | 57. 0483 |
| 8. 63 | 44, 184 | 92. 6319 | 24618. 1826 | 56. 6754 |
| 8. 64 | 43, 776 | 92. 4635 | 24573. 4142 | 56. 3029 |
| 8. 64 | 43, 372 | 92. 2870 | 24526. 5159 | 55. 9309 |
| 8. 65 | 42, 971 | 92. 0672 | 24468. 1017 | 55. 5595 |
| 8. 65 | 42, 574 | 91. 7953 | 24395. 8468 | 55. 1890 |
| 8. 66 | 42, 180 | 91. 4891 | 24314. 4650 | 54. 8194 |
| 8. 66 | 41, 789 | 91. 1662 | 24228. 6578 | 54. 4510 |
| 8. 67 | 41, 402 | 90. 8620 | 24147. 7974 | 54. 0836 |
| 8. 67 | 41, 018 | 90. 5851 | 24074. 2132 | 53. 7170 |
| 8. 68 | 40, 637 | 90. 3355 | 24007. 8905 | 53. 3513 |
| 8. 68 | 40, 260 | 90. 0605 | 23934. 7915 | 52. 9864 |
| 8. 69 | 39, 886 | 89. 8215 | 23871. 2778 | 52. 6223 |
| 8. 69 | 39, 516 | 89. 5131 | 23789. 3177 | 52. 2592 |
| 8. 70 | 39, 148 | 89. 1179 | 23684. 2756 | 51. 8975 |
| 8. 70 | 38, 784 | 88. 7236 | 23579. 5072 | 51. 5373 |
| 8. 71 | 38, 423 | 88. 3305 | 23475. 0100 | 51. 1784 |
| 8. 71 | 38, 065 | 87. 9383 | 23370. 7815 | 50. 8209 |
| 8. 72 | 37, 710 | 87. 5734 | 23273. 8029 | 50. 4647 |
| 8. 72 | 37, 359 | 87. 2532 | 23188. 7136 | 50. 1096 |
| 8. 73 | 37, 010 | 86. 9252 | 23101. 5404 | 49. 7556 |
| 8. 73 | 36, 665 | 86. 5981 | 23014. 6091 | 49. 4028 |
| 8. 74 | 36, 322 | 86. 2107 | 22911. 6570 | 49. 0514 |
| 8. 74 | 35, 983 | 85. 7369 | 22785. 7421 | 48. 7018 |
| 8. 75 | 35, 646 | 85. 2904 | 22667. 0646 | 48. 3537 |
| 8. 75 | 35, 313 | 84. 8448 | 22548. 6531 | 48. 0074 |
| 8. 76 | 34, 982 | 84. 3828 | 22425. 8684 | 47. 6627 |
| 8. 76 | 34, 655 | 83. 9567 | 22312. 6172 | 47. 3196 |
| 8. 77 | 34, 330 | 83. 6186 | 22222. 7793 | 46. 9778 |
| 8. 77 | 34, 008 | 83. 2466 | 22123. 9107 | 46. 6373 |
| 8. 78 | 33, 690 | 82. 8581 | 22020. 6500 | 46. 2982 |
| 8. 78 | 33, 374 | 82. 3834 | 21894. 4995 | 45. 9609 |
| 8. 79 | 33, 060 | 81. 9358 | 21775. 5403 | 45. 6253 |
| 8. 79 | 32, 750 | 81. 4022 | 21633. 7221 | 45. 2917 |
| 8. 80 | 32, 442 | 80. 9043 | 21501. 4074 | 44. 9600 |
| 8. 80 | 32, 137 | 80. 4161 | 21371. 6553 | 44. 6302 |
| 8. 81 | 31, 835 | 80. 0243 | 21267. 5362 | 44. 3018 |

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| 8. 81 | 31, 536 | 79. 6420 | 21165. 9486 | 43. 9749 |
| 8. 82 | 31, 239 | 79. 1999 | 21048. 4419 | 43. 6497 |
| 8. 82 | 30, 945 | 78. 7326 | 20924. 2527 | 43. 3262 |
| 8. 83 | 30, 654 | 78. 2835 | 20804. 9058 | 43. 0045 |
| 8. 83 | 30, 365 | 77. 8440 | 20688. 0902 | 42. 6844 |
| 8. 84 | 30, 079 | 77. 3100 | 20546. 1767 | 42. 3664 |
| 8. 84 | 29, 795 | 76. 7856 | 20406. 8053 | 42. 0504 |
| 8. 85 | 29, 514 | 76. 2967 | 20276. 8711 | 41. 7364 |
| 8. 85 | 29, 236 | 75. 8951 | 20170. 1573 | 41. 4238 |
| 8. 86 | 28, 960 | 75. 4771 | 20059. 0535 | 41. 1129 |
| 8. 86 | 28, 686 | 75. 0252 | 19938. 9668 | 40. 8038 |
| 8. 87 | 28, 415 | 74. 5655 | 19816. 7953 | 40. 4964 |
| 8. 87 | 28, 147 | 74. 1066 | 19694. 8350 | 40. 1908 |
| 8. 88 | 27, 881 | 73. 6658 | 19577. 6741 | 39. 8869 |
| 8. 88 | 27, 617 | 73. 1307 | 19435. 4757 | 39. 5851 |
| 8. 89 | 27, 356 | 72. 5792 | 19288. 9066 | 39. 2855 |
| 8. 89 | 27, 097 | 72. 1321 | 19170. 0721 | 38. 9877 |
| 8. 90 | 26, 841 | 71. 7202 | 19060. 6042 | 38. 6914 |
| 8. 90 | 26, 586 | 71. 2831 | 18944. 4499 | 38. 3969 |
| 8. 91 | 26, 335 | 70. 7864 | 18812. 4492 | 38. 1044 |
| 8. 91 | 26, 085 | 70. 3594 | 18698. 9690 | 37. 8135 |
| 8. 92 | 25, 838 | 69. 9245 | 18583. 3836 | 37. 5243 |
| 8. 92 | 25, 593 | 69. 4558 | 18458. 8254 | 37. 2370 |
| 8. 93 | 25, 350 | 68. 9534 | 18325. 2997 | 36. 9517 |
| 8. 93 | 25, 110 | 68. 4431 | 18189. 6745 | 36. 6685 |
| 8. 94 | 24, 872 | 68. 0109 | 18074. 8215 | 36. 3869 |
| 8. 94 | 24, 636 | 67. 5622 | 17955. 5690 | 36. 1071 |
| 8. 95 | 24, 402 | 67. 0711 | 17825. 0598 | 35. 8293 |
| 8. 95 | 24, 170 | 66. 6495 | 17713. 0118 | 35. 5532 |
| 8. 96 | 23, 941 | 66. 2285 | 17601. 1295 | 35. 2788 |
| 8. 96 | 23, 714 | 65. 8168 | 17491. 6950 | 35. 0060 |
| 8. 97 | 23, 488 | 65. 3283 | 17361. 8651 | 34. 7352 |
| 8. 97 | 23, 265 | 64. 8318 | 17229. 9185 | 34. 4663 |
| 8. 98 | 23, 044 | 64. 3359 | 17098. 1372 | 34. 1995 |
| 8. 98 | 22, 825 | 63. 9008 | 16982. 4963 | 33. 9344 |
| 8. 99 | 22, 608 | 63. 4233 | 16855. 5987 | 33. 6713 |
| 8. 99 | 22, 393 | 62. 9807 | 16737. 9836 | 33. 4099 |
| 9. 00 | 22, 180 | 62. 5473 | 16622. 7996 | 33. 1503 |
| 9. 00 | 21, 969 | 62. 1059 | 16505. 4817 | 32. 8924 |
| 9. 01 | 21, 760 | 61. 6221 | 16376. 9069 | 32. 6366 |
| 9. 01 | 21, 553 | 61. 1732 | 16257. 5992 | 32. 3825 |
| 9. 02 | 21, 348 | 60. 7334 | 16140. 7126 | 32. 1303 |
| 9. 02 | 21, 145 | 60. 2769 | 16019. 4057 | 31. 8799 |
| 9. 03 | 20, 943 | 59. 7953 | 15891. 3992 | 31. 6315 |
| 9. 03 | 20, 744 | 59. 3570 | 15774. 9231 | 31. 3848 |
| 9. 04 | 20, 546 | 58. 9449 | 15665. 4138 | 31. 1398 |
| 9. 04 | 20, 351 | 58. 5076 | 15549. 1976 | 30. 8967 |
| 9. 05 | 20, 157 | 58. 0708 | 15433. 1084 | 30. 6553 |
| 9. 05 | 19, 965 | 57. 6002 | 15308. 0337 | 30. 4158 |
| 9. 06 | 19, 774 | 57. 1729 | 15194. 4701 | 30. 1781 |
| 9. 06 | 19, 586 | 56. 7632 | 15085. 5805 | 29. 9421 |
| 9. 07 | 19, 399 | 56. 3196 | 14967. 7000 | 29. 7079 |
| 9. 07 | 19, 215 | 55. 8851 | 14852. 2124 | 29. 4754 |
| 9. 08 | 19, 031 | 55. 4509 | 14736. 8385 | 29. 2448 |
| 9. 08 | 18, 850 | 55. 0258 | 14623. 8522 | 29. 0159 |
| 9. 09 | 18, 670 | 54. 5668 | 14501. 8701 | 28. 7889 |
| 9. 09 | 18, 492 | 54. 1511 | 14391. 3760 | 28. 5636 |
| 9. 10 | 18, 316 | 53. 7271 | 14278. 7101 | 28. 3401 |
| 9. 10 | 18, 141 | 53. 3121 | 14168. 4224 | 28. 1183 |
| 9. 11 | 17, 968 | 52. 8804 | 14053. 6833 | 27. 8983 |
| 9. 11 | 17, 797 | 52. 4747 | 13945. 8692 | 27. 6799 |
| 9. 12 | 17, 627 | 52. 0352 | 13829. 0486 | 27. 4634 |
| 9. 12 | 17, 459 | 51. 6302 | 13721. 4239 | 27. 2486 |
| 9. 13 | 17, 293 | 51. 1828 | 13602. 5136 | 27. 0356 |
| 9. 13 | 17, 128 | 50. 7528 | 13488. 2437 | 26. 8244 |
| 9. 14 | 16, 964 | 50. 3232 | 13374. 0612 | 26. 6150 |
| 9. 14 | 16, 803 | 49. 9110 | 13264. 5147 | 26. 4073 |
| 9. 15 | 16, 642 | 49. 4991 | 13155. 0513 | 26. 2013 |
| 9. 15 | 16, 484 | 49. 0961 | 13047. 9444 | 25. 9970 |
| 9. 16 | 16, 327 | 48. 7190 | 12947. 7429 | 25. 7942 |

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| 9. 16 | 16. 171 | 48. 3166 | 12840. 7922 | 25. 5932 |
| 9. 17 | 16. 017 | 47. 8973 | 12729. 3654 | 25. 3939 |
| 9. 17 | 15. 864 | 47. 4698 | 12615. 7356 | 25. 1964 |
| 9. 18 | 15. 713 | 47. 0425 | 12502. 1762 | 25. 0007 |
| 9. 18 | 15. 563 | 46. 6754 | 12404. 6163 | 24. 8065 |
| 9. 19 | 15. 415 | 46. 2743 | 12298. 0202 | 24. 6140 |
| 9. 19 | 15. 268 | 45. 8735 | 12191. 4891 | 24. 4231 |
| 9. 20 | 15. 122 | 45. 5414 | 12103. 2314 | 24. 2337 |
| 9. 20 | 14. 978 | 45. 2266 | 12019. 5905 | 24. 0456 |
| 9. 21 | 14. 835 | 44. 8522 | 11920. 0755 | 23. 8590 |
| 9. 21 | 14. 694 | 44. 4094 | 11802. 4038 | 23. 6744 |
| 9. 22 | 14. 554 | 43. 9754 | 11687. 0623 | 23. 4915 |
| 9. 22 | 14. 415 | 43. 6102 | 11589. 9907 | 23. 3102 |
| 9. 23 | 14. 278 | 43. 2280 | 11488. 4170 | 23. 1305 |
| 9. 23 | 14. 142 | 42. 8288 | 11382. 3378 | 22. 9524 |
| 9. 24 | 14. 007 | 42. 4899 | 11292. 2526 | 22. 7758 |
| 9. 24 | 13. 874 | 42. 1768 | 11209. 0517 | 22. 6005 |
| 9. 25 | 13. 741 | 41. 9068 | 11137. 2937 | 22. 4264 |
| 9. 25 | 13. 611 | 41. 5341 | 11038. 2385 | 22. 2538 |
| 9. 26 | 13. 481 | 41. 1186 | 10927. 8282 | 22. 0830 |
| 9. 26 | 13. 353 | 40. 7290 | 10824. 2933 | 21. 9139 |
| 9. 27 | 13. 226 | 40. 3739 | 10729. 9150 | 21. 7462 |
| 9. 27 | 13. 100 | 40. 0018 | 10631. 0134 | 21. 5801 |
| 9. 28 | 12. 975 | 39. 6383 | 10534. 4267 | 21. 4156 |
| 9. 28 | 12. 852 | 39. 3523 | 10458. 4041 | 21. 2522 |
| 9. 29 | 12. 729 | 39. 1179 | 10396. 1101 | 21. 0899 |
| 9. 29 | 12. 608 | 38. 8150 | 10315. 6021 | 20. 9288 |
| 9. 30 | 12. 488 | 38. 4778 | 10225. 9984 | 20. 7692 |
| 9. 30 | 12. 370 | 38. 1064 | 10127. 2917 | 20. 6112 |
| 9. 31 | 12. 252 | 37. 7780 | 10040. 0258 | 20. 4545 |
| 9. 31 | 12. 136 | 37. 4240 | 9945. 9347 | 20. 2994 |
| 9. 32 | 12. 020 | 37. 0529 | 9847. 2966 | 20. 1458 |
| 9. 32 | 11. 906 | 36. 7506 | 9766. 9565 | 19. 9935 |
| 9. 33 | 11. 793 | 36. 4999 | 9700. 3542 | 19. 8423 |
| 9. 33 | 11. 681 | 36. 2667 | 9638. 3539 | 19. 6921 |
| 9. 34 | 11. 570 | 35. 9905 | 9564. 9513 | 19. 5430 |
| 9. 34 | 11. 461 | 35. 7230 | 9493. 8599 | 19. 3951 |
| 9. 35 | 11. 352 | 35. 4039 | 9409. 0654 | 19. 2486 |
| 9. 35 | 11. 244 | 35. 0591 | 9317. 4206 | 19. 1036 |
| 9. 36 | 11. 138 | 34. 7315 | 9230. 3637 | 18. 9599 |
| 9. 36 | 11. 032 | 34. 4298 | 9150. 1863 | 18. 8175 |
| 9. 37 | 10. 928 | 34. 1713 | 9081. 4735 | 18. 6762 |
| 9. 37 | 10. 824 | 33. 9645 | 9026. 5248 | 18. 5359 |
| 9. 38 | 10. 722 | 33. 7406 | 8967. 0176 | 18. 3965 |
| 9. 38 | 10. 620 | 33. 4823 | 8898. 3619 | 18. 2582 |
| 9. 39 | 10. 520 | 33. 2412 | 8834. 3067 | 18. 1209 |
| 9. 39 | 10. 420 | 32. 9657 | 8761. 0921 | 17. 9848 |
| 9. 40 | 10. 321 | 32. 6126 | 8667. 2348 | 17. 8502 |
| 9. 40 | 10. 224 | 32. 3026 | 8584. 8479 | 17. 7170 |
| 9. 41 | 10. 127 | 32. 0185 | 8509. 3490 | 17. 5850 |
| 9. 41 | 10. 031 | 31. 8295 | 8459. 1189 | 17. 4537 |
| 9. 42 | 9. 937 | 31. 6319 | 8406. 6102 | 17. 3234 |
| 9. 42 | 9. 843 | 31. 3998 | 8344. 9237 | 17. 1940 |
| 9. 43 | 9. 750 | 31. 1850 | 8287. 8447 | 17. 0656 |
| 9. 43 | 9. 658 | 30. 9790 | 8233. 0770 | 16. 9381 |
| 9. 44 | 9. 567 | 30. 7296 | 8166. 8155 | 16. 8117 |
| 9. 44 | 9. 477 | 30. 4283 | 8086. 7440 | 16. 6865 |
| 9. 45 | 9. 387 | 30. 1703 | 8018. 1766 | 16. 5624 |
| 9. 45 | 9. 299 | 29. 9123 | 7949. 6065 | 16. 4395 |
| 9. 46 | 9. 211 | 29. 7324 | 7901. 7749 | 16. 3173 |
| 9. 46 | 9. 125 | 29. 5524 | 7853. 9551 | 16. 1960 |
| 9. 47 | 9. 039 | 29. 3552 | 7801. 5327 | 16. 0755 |
| 9. 47 | 8. 954 | 29. 1666 | 7751. 4240 | 15. 9558 |
| 9. 48 | 8. 870 | 28. 9607 | 7696. 7051 | 15. 8370 |
| 9. 48 | 8. 786 | 28. 7114 | 7630. 4402 | 15. 7193 |
| 9. 49 | 8. 704 | 28. 4533 | 7561. 8554 | 15. 6027 |
| 9. 49 | 8. 622 | 28. 2039 | 7495. 5697 | 15. 4872 |
| 9. 50 | 8. 541 | 27. 9980 | 7440. 8355 | 15. 3726 |
| 9. 50 | 8. 461 | 27. 8094 | 7390. 7265 | 15. 2588 |
| 9. 51 | 8. 382 | 27. 6557 | 7349. 8777 | 15. 1456 |

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| 9. 51 | 8. 303 | 27. 4759 | 7302. 0897 | 15. 0333 |
| 9. 52 | 8. 225 | 27. 2961 | 7254. 3028 | 14. 9217 |
| 9. 52 | 8. 148 | 27. 1076 | 7204. 1980 | 14. 8110 |
| 9. 53 | 8. 072 | 26. 9103 | 7151. 7709 | 14. 7012 |
| 9. 53 | 7. 996 | 26. 6955 | 7094. 6965 | 14. 5922 |
| 9. 54 | 7. 922 | 26. 4458 | 7028. 3232 | 14. 4844 |
| 9. 54 | 7. 848 | 26. 2659 | 6980. 5104 | 14. 3773 |
| 9. 55 | 7. 774 | 26. 0859 | 6932. 6930 | 14. 2710 |
| 9. 55 | 7. 702 | 25. 9410 | 6894. 1714 | 14. 1654 |
| 9. 56 | 7. 630 | 25. 8048 | 6857. 9799 | 14. 0604 |
| 9. 56 | 7. 559 | 25. 6249 | 6810. 1544 | 13. 9562 |
| 9. 57 | 7. 488 | 25. 4274 | 6757. 6624 | 13. 8528 |
| 9. 57 | 7. 419 | 25. 2473 | 6709. 8170 | 13. 7502 |
| 9. 58 | 7. 349 | 25. 0673 | 6661. 9614 | 13. 6484 |
| 9. 58 | 7. 281 | 24. 8345 | 6600. 0962 | 13. 5476 |
| 9. 59 | 7. 213 | 24. 6543 | 6552. 2094 | 13. 4476 |
| 9. 59 | 7. 146 | 24. 4916 | 6508. 9811 | 13. 3483 |
| 9. 60 | 7. 080 | 24. 3465 | 6470. 4185 | 13. 2497 |
| 9. 60 | 7. 014 | 24. 1662 | 6422. 4980 | 13. 1519 |
| 9. 61 | 6. 949 | 23. 9858 | 6374. 5615 | 13. 0548 |
| 9. 61 | 6. 885 | 23. 8318 | 6333. 6325 | 12. 9584 |
| 9. 62 | 6. 821 | 23. 6602 | 6288. 0086 | 12. 8628 |
| 9. 62 | 6. 758 | 23. 5149 | 6249. 4019 | 12. 7678 |
| 9. 63 | 6. 695 | 23. 3078 | 6194. 3669 | 12. 6737 |
| 9. 63 | 6. 633 | 23. 1536 | 6153. 3860 | 12. 5803 |
| 9. 64 | 6. 572 | 22. 9994 | 6112. 3925 | 12. 4876 |
| 9. 64 | 6. 511 | 22. 8628 | 6076. 0865 | 12. 3955 |
| 9. 65 | 6. 451 | 22. 6819 | 6028. 0130 | 12. 3042 |
| 9. 65 | 6. 392 | 22. 5452 | 5991. 6824 | 12. 2135 |
| 9. 66 | 6. 333 | 22. 3907 | 5950. 6322 | 12. 1235 |
| 9. 66 | 6. 274 | 22. 2362 | 5909. 5654 | 12. 0341 |
| 9. 67 | 6. 217 | 22. 0727 | 5866. 1228 | 11. 9455 |
| 9. 67 | 6. 159 | 21. 9092 | 5822. 6587 | 11. 8576 |
| 9. 68 | 6. 103 | 21. 7811 | 5788. 6182 | 11. 7703 |
| 9. 68 | 6. 047 | 21. 6619 | 5756. 9308 | 11. 6835 |
| 9. 69 | 5. 991 | 21. 4981 | 5713. 4116 | 11. 5974 |
| 9. 69 | 5. 936 | 21. 3432 | 5672. 2339 | 11. 5120 |
| 9. 70 | 5. 882 | 21. 1703 | 5626. 2970 | 11. 4274 |
| 9. 70 | 5. 828 | 21. 0509 | 5594. 5503 | 11. 3433 |
| 9. 71 | 5. 775 | 20. 8689 | 5546. 1904 | 11. 2600 |
| 9. 71 | 5. 722 | 20. 7672 | 5519. 1575 | 11. 1771 |
| 9. 72 | 5. 669 | 20. 6565 | 5489. 7447 | 11. 0948 |
| 9. 72 | 5. 618 | 20. 5369 | 5457. 9460 | 11. 0130 |
| 9. 73 | 5. 566 | 20. 3634 | 5411. 8599 | 10. 9319 |
| 9. 73 | 5. 516 | 20. 2078 | 5370. 4999 | 10. 8516 |
| 9. 74 | 5. 465 | 20. 0431 | 5326. 7277 | 10. 7719 |
| 9. 74 | 5. 416 | 19. 9501 | 5301. 9993 | 10. 6927 |
| 9. 75 | 5. 366 | 19. 7672 | 5253. 4003 | 10. 6143 |
| 9. 75 | 5. 318 | 19. 6291 | 5216. 7006 | 10. 5365 |
| 9. 76 | 5. 269 | 19. 5449 | 5194. 3189 | 10. 4591 |
| 9. 76 | 5. 221 | 19. 4877 | 5179. 1124 | 10. 3819 |
| 9. 77 | 5. 174 | 19. 3314 | 5137. 5760 | 10. 3055 |
| 9. 77 | 5. 127 | 19. 1930 | 5100. 7975 | 10. 2297 |
| 9. 78 | 5. 081 | 19. 0365 | 5059. 1939 | 10. 1545 |
| 9. 78 | 5. 035 | 18. 8978 | 5022. 3545 | 10. 0800 |
| 9. 79 | 4. 989 | 18. 7681 | 4987. 8880 | 10. 0060 |
| 9. 79 | 4. 944 | 18. 6474 | 4955. 7999 | 9. 9326 |
| 9. 80 | 4. 900 | 18. 5175 | 4921. 2822 | 9. 8597 |
| 9. 80 | 4. 856 | 18. 4782 | 4910. 8259 | 9. 7871 |
| 9. 81 | 4. 812 | 18. 4026 | 4890. 7397 | 9. 7148 |
| 9. 81 | 4. 768 | 18. 2725 | 4856. 1709 | 9. 6431 |
| 9. 82 | 4. 726 | 18. 1423 | 4821. 5724 | 9. 5720 |
| 9. 82 | 4. 683 | 18. 0120 | 4786. 9433 | 9. 5015 |
| 9. 83 | 4. 641 | 17. 8907 | 4754. 7026 | 9. 4315 |
| 9. 83 | 4. 600 | 17. 7602 | 4720. 0123 | 9. 3620 |
| 9. 84 | 4. 558 | 17. 6204 | 4682. 8647 | 9. 2932 |
| 9. 84 | 4. 518 | 17. 5170 | 4655. 3854 | 9. 2249 |
| 9. 85 | 4. 477 | 17. 4775 | 4644. 8880 | 9. 1567 |
| 9. 85 | 4. 437 | 17. 4198 | 4629. 5395 | 9. 0889 |
| 9. 86 | 4. 398 | 17. 2979 | 4597. 1572 | 9. 0216 |

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|--------|--------|----------|------------|---------|
| 9. 86 | 4. 358 | 17. 1668 | 4562. 3072 | 8. 9549 |
| 9. 87 | 4. 320 | 17. 0355 | 4527. 4200 | 8. 8887 |
| 9. 87 | 4. 281 | 16. 8949 | 4490. 0538 | 8. 8232 |
| 9. 88 | 4. 243 | 16. 7634 | 4455. 0870 | 8. 7582 |
| 9. 88 | 4. 205 | 16. 6868 | 4434. 7546 | 8. 6936 |
| 9. 89 | 4. 168 | 16. 6748 | 4431. 5484 | 8. 6291 |
| 9. 89 | 4. 131 | 16. 6444 | 4423. 4646 | 8. 5648 |
| 9. 90 | 4. 095 | 16. 5863 | 4408. 0347 | 8. 5008 |
| 9. 90 | 4. 058 | 16. 5005 | 4385. 2359 | 8. 4371 |
| 9. 91 | 4. 022 | 16. 3407 | 4342. 7532 | 8. 3742 |
| 9. 91 | 3. 987 | 16. 1991 | 4305. 1329 | 8. 3119 |
| 9. 92 | 3. 952 | 16. 0388 | 4262. 5356 | 8. 2502 |
| 9. 92 | 3. 917 | 15. 9433 | 4237. 1395 | 8. 1890 |
| 9. 93 | 3. 883 | 15. 8383 | 4209. 2492 | 8. 1283 |
| 9. 93 | 3. 848 | 15. 8263 | 4206. 0420 | 8. 0676 |
| 9. 94 | 3. 815 | 15. 7863 | 4195. 4342 | 8. 0072 |
| 9. 94 | 3. 781 | 15. 7278 | 4179. 8783 | 7. 9471 |
| 9. 95 | 3. 748 | 15. 6693 | 4164. 3168 | 7. 8873 |
| 9. 95 | 3. 715 | 15. 5266 | 4126. 4080 | 7. 8280 |
| 9. 96 | 3. 683 | 15. 3651 | 4083. 4723 | 7. 7695 |
| 9. 96 | 3. 651 | 15. 2688 | 4057. 8837 | 7. 7114 |
| 9. 97 | 3. 619 | 15. 1630 | 4029. 7742 | 7. 6537 |
| 9. 97 | 3. 587 | 15. 1134 | 4016. 5902 | 7. 5964 |
| 9. 98 | 3. 556 | 15. 0544 | 4000. 9071 | 7. 5393 |
| 9. 98 | 3. 525 | 15. 0047 | 3987. 7148 | 7. 4824 |
| 9. 99 | 3. 495 | 14. 9833 | 3982. 0265 | 7. 4257 |
| 9. 99 | 3. 464 | 14. 9243 | 3966. 3311 | 7. 3693 |
| 10. 00 | 3. 434 | 14. 7897 | 3930. 5591 | 7. 3135 |
| 10. 00 | 3. 405 | 14. 6643 | 3897. 2404 | 7. 2582 |
| 10. 01 | 3. 375 | 14. 5482 | 3866. 3831 | 7. 2034 |
| 10. 01 | 3. 346 | 14. 4224 | 3832. 9605 | 7. 1492 |
| 10. 02 | 3. 317 | 14. 3724 | 3819. 6473 | 7. 0952 |
| 10. 02 | 3. 289 | 14. 3602 | 3816. 4228 | 7. 0413 |
| 10. 03 | 3. 260 | 14. 3006 | 3800. 5832 | 6. 9877 |
| 10. 03 | 3. 232 | 14. 2695 | 3792. 3205 | 6. 9343 |
| 10. 04 | 3. 205 | 14. 2099 | 3776. 4645 | 6. 8812 |
| 10. 04 | 3. 177 | 14. 1406 | 3758. 0586 | 6. 8284 |
| 10. 05 | 3. 150 | 14. 0044 | 3721. 8555 | 6. 7762 |
| 10. 05 | 3. 123 | 13. 9062 | 3695. 7571 | 6. 7244 |
| 10. 06 | 3. 096 | 13. 7695 | 3659. 4331 | 6. 6732 |
| 10. 06 | 3. 070 | 13. 7477 | 3653. 6338 | 6. 6221 |
| 10. 07 | 3. 044 | 13. 7163 | 3645. 2925 | 6. 5713 |
| 10. 07 | 3. 018 | 13. 7138 | 3644. 6235 | 6. 5205 |
| 10. 08 | 2. 992 | 13. 6439 | 3626. 0619 | 6. 4700 |
| 10. 08 | 2. 967 | 13. 6029 | 3615. 1654 | 6. 4197 |
| 10. 09 | 2. 942 | 13. 5619 | 3604. 2654 | 6. 3697 |
| 10. 09 | 2. 917 | 13. 4242 | 3567. 6627 | 6. 3202 |
| 10. 10 | 2. 892 | 13. 2668 | 3525. 8392 | 6. 2714 |
| 10. 10 | 2. 868 | 13. 2158 | 3512. 2725 | 6. 2229 |
| 10. 11 | 2. 844 | 13. 1841 | 3503. 8545 | 6. 1745 |
| 10. 11 | 2. 820 | 13. 1719 | 3500. 6065 | 6. 1262 |
| 10. 12 | 2. 796 | 13. 1403 | 3492. 1991 | 6. 0781 |
| 10. 12 | 2. 773 | 13. 0696 | 3473. 4278 | 6. 0304 |
| 10. 13 | 2. 750 | 13. 0086 | 3457. 2235 | 5. 9829 |
| 10. 13 | 2. 727 | 12. 9672 | 3446. 1953 | 5. 9357 |
| 10. 14 | 2. 704 | 12. 8277 | 3409. 1357 | 5. 8890 |
| 10. 14 | 2. 681 | 12. 6879 | 3371. 9938 | 5. 8429 |
| 10. 15 | 2. 659 | 12. 6657 | 3366. 0888 | 5. 7969 |
| 10. 15 | 2. 637 | 12. 6829 | 3370. 6464 | 5. 7510 |
| 10. 16 | 2. 615 | 12. 6509 | 3362. 1534 | 5. 7052 |
| 10. 16 | 2. 593 | 12. 5992 | 3348. 4164 | 5. 6597 |
| 10. 17 | 2. 572 | 12. 5475 | 3334. 6629 | 5. 6145 |
| 10. 17 | 2. 551 | 12. 5352 | 3331. 4104 | 5. 5693 |
| 10. 18 | 2. 530 | 12. 4933 | 3320. 2720 | 5. 5244 |
| 10. 18 | 2. 509 | 12. 3720 | 3288. 0245 | 5. 4800 |
| 10. 19 | 2. 488 | 12. 2603 | 3258. 3440 | 5. 4361 |
| 10. 19 | 2. 468 | 12. 2380 | 3252. 4062 | 5. 3923 |
| 10. 20 | 2. 447 | 12. 2356 | 3251. 7733 | 5. 3485 |
| 10. 20 | 2. 427 | 12. 1734 | 3235. 2395 | 5. 3051 |
| 10. 21 | 2. 407 | 12. 1311 | 3223. 9938 | 5. 2619 |

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|--------|--------|----------|------------|---------|
| 10. 21 | 2. 388 | 12. 0687 | 3207. 4119 | 5. 2190 |
| 10. 22 | 2. 368 | 12. 0262 | 3196. 1340 | 5. 1763 |
| 10. 22 | 2. 349 | 11. 9637 | 3179. 5024 | 5. 1338 |
| 10. 23 | 2. 330 | 11. 9010 | 3162. 8409 | 5. 0917 |
| 10. 23 | 2. 311 | 11. 8482 | 3148. 8284 | 5. 0498 |
| 10. 24 | 2. 292 | 11. 8257 | 3142. 8448 | 5. 0081 |
| 10. 24 | 2. 274 | 11. 7830 | 3131. 4889 | 4. 9666 |
| 10. 25 | 2. 255 | 11. 7402 | 3120. 1189 | 4. 9253 |
| 10. 25 | 2. 237 | 11. 7075 | 3111. 4316 | 4. 8842 |
| 10. 26 | 2. 219 | 11. 6850 | 3105. 4402 | 4. 8433 |
| 10. 26 | 2. 201 | 11. 6115 | 3085. 9216 | 4. 8026 |
| 10. 27 | 2. 184 | 11. 5685 | 3074. 4923 | 4. 7622 |
| 10. 27 | 2. 166 | 11. 5459 | 3068. 4778 | 4. 7220 |
| 10. 28 | 2. 149 | 11. 5028 | 3057. 0261 | 4. 6819 |
| 10. 28 | 2. 132 | 11. 4186 | 3034. 6595 | 4. 6422 |
| 10. 29 | 2. 115 | 11. 3548 | 3017. 6973 | 4. 6029 |
| 10. 29 | 2. 098 | 11. 3011 | 3003. 4329 | 4. 5637 |
| 10. 30 | 2. 081 | 11. 2680 | 2994. 6193 | 4. 5248 |
| 10. 30 | 2. 065 | 11. 2038 | 2977. 5661 | 4. 4861 |
| 10. 31 | 2. 049 | 11. 1395 | 2960. 4751 | 4. 4477 |
| 10. 31 | 2. 032 | 11. 1269 | 2957. 1135 | 4. 4095 |
| 10. 32 | 2. 016 | 11. 1143 | 2953. 7625 | 4. 3713 |
| 10. 32 | 2. 001 | 11. 1017 | 2950. 4220 | 4. 3333 |
| 10. 33 | 1. 985 | 11. 0371 | 2933. 2494 | 4. 2955 |
| 10. 33 | 1. 969 | 10. 9932 | 2921. 5838 | 4. 2580 |
| 10. 34 | 1. 954 | 10. 9806 | 2918. 2339 | 4. 2206 |
| 10. 34 | 1. 939 | 10. 9680 | 2914. 8943 | 4. 1833 |
| 10. 35 | 1. 923 | 10. 8820 | 2892. 0412 | 4. 1463 |
| 10. 35 | 1. 909 | 10. 8378 | 2880. 3022 | 4. 1096 |
| 10. 36 | 1. 894 | 10. 8357 | 2879. 7397 | 4. 0730 |
| 10. 36 | 1. 879 | 10. 8231 | 2876. 3925 | 4. 0364 |
| 10. 37 | 1. 864 | 10. 8106 | 2873. 0551 | 4. 0000 |
| 10. 37 | 1. 850 | 10. 7769 | 2864. 0959 | 3. 9638 |
| 10. 38 | 1. 836 | 10. 7325 | 2852. 3035 | 3. 9277 |
| 10. 38 | 1. 822 | 10. 7306 | 2851. 7941 | 3. 8918 |
| 10. 39 | 1. 808 | 10. 6754 | 2837. 1444 | 3. 8561 |
| 10. 39 | 1. 794 | 10. 6309 | 2825. 2972 | 3. 8206 |
| 10. 40 | 1. 780 | 10. 5969 | 2816. 2687 | 3. 7853 |
| 10. 40 | 1. 767 | 10. 5629 | 2807. 2267 | 3. 7502 |
| 10. 41 | 1. 753 | 10. 5825 | 2812. 4443 | 3. 7151 |
| 10. 41 | 1. 740 | 10. 6023 | 2817. 7052 | 3. 6800 |
| 10. 42 | 1. 727 | 10. 5360 | 2800. 0796 | 3. 6452 |
| 10. 42 | 1. 713 | 10. 4911 | 2788. 1488 | 3. 6106 |
| 10. 43 | 1. 701 | 10. 4244 | 2770. 4353 | 3. 5763 |
| 10. 43 | 1. 688 | 10. 3467 | 2749. 7870 | 3. 5424 |
| 10. 44 | 1. 675 | 10. 3123 | 2740. 6350 | 3. 5086 |
| 10. 44 | 1. 662 | 10. 2996 | 2737. 2588 | 3. 4749 |
| 10. 45 | 1. 650 | 10. 2869 | 2733. 8905 | 3. 4414 |
| 10. 45 | 1. 638 | 10. 3399 | 2747. 9795 | 3. 4077 |
| 10. 46 | 1. 625 | 10. 3823 | 2759. 2361 | 3. 3740 |
| 10. 46 | 1. 613 | 10. 3480 | 2750. 1186 | 3. 3404 |
| 10. 47 | 1. 601 | 10. 2586 | 2726. 3495 | 3. 3072 |
| 10. 47 | 1. 589 | 10. 2130 | 2714. 2329 | 3. 2743 |
| 10. 48 | 1. 578 | 10. 1894 | 2707. 9652 | 3. 2414 |
| 10. 48 | 1. 566 | 10. 1547 | 2698. 7461 | 3. 2088 |
| 10. 49 | 1. 554 | 10. 1422 | 2695. 4137 | 3. 1763 |
| 10. 49 | 1. 543 | 10. 1185 | 2689. 1296 | 3. 1439 |
| 10. 50 | 1. 532 | 10. 1841 | 2706. 5658 | 3. 1114 |
| 10. 50 | 1. 521 | 10. 2389 | 2721. 1300 | 3. 0788 |
| 10. 51 | 1. 509 | 10. 2268 | 2717. 9075 | 3. 0463 |
| 10. 51 | 1. 498 | 10. 1023 | 2684. 8340 | 3. 0142 |
| 10. 52 | 1. 488 | 10. 0225 | 2663. 6028 | 2. 9825 |
| 10. 52 | 1. 477 | 9. 9874 | 2654. 2947 | 2. 9510 |
| 10. 53 | 1. 466 | 9. 9750 | 2650. 9790 | 2. 9196 |
| 10. 53 | 1. 455 | 9. 9398 | 2641. 6429 | 2. 8883 |
| 10. 54 | 1. 445 | 9. 9955 | 2656. 4501 | 2. 8570 |
| 10. 54 | 1. 435 | 10. 0744 | 2677. 4045 | 2. 8255 |
| 10. 55 | 1. 424 | 10. 1537 | 2698. 4805 | 2. 7938 |
| 10. 55 | 1. 414 | 10. 1190 | 2689. 2587 | 2. 7623 |
| 10. 56 | 1. 404 | 10. 0383 | 2667. 8180 | 2. 7312 |

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|-------|-------|---------|-----------|--------|
| 10.56 | 1.394 | 9.9688 | 2649.3536 | 2.7003 |
| 10.57 | 1.384 | 9.9568 | 2646.1429 | 2.6695 |
| 10.57 | 1.374 | 9.8869 | 2627.5812 | 2.6390 |
| 10.58 | 1.365 | 9.8747 | 2624.3468 | 2.6087 |
| 10.58 | 1.355 | 9.9439 | 2642.7260 | 2.5782 |
| 10.59 | 1.345 | 10.0484 | 2670.5022 | 2.5474 |
| 10.59 | 1.336 | 10.0952 | 2682.9293 | 2.5166 |
| 10.60 | 1.327 | 10.1189 | 2689.2220 | 2.4858 |
| 10.60 | 1.317 | 10.0372 | 2667.5098 | 2.4553 |
| 10.61 | 1.308 | 9.9787 | 2651.9619 | 2.4251 |
| 10.61 | 1.299 | 9.8964 | 2630.0903 | 2.3952 |
| 10.62 | 1.290 | 9.8137 | 2608.1292 | 2.3656 |
| 10.62 | 1.281 | 9.8374 | 2614.4266 | 2.3360 |
| 10.63 | 1.272 | 9.8851 | 2627.0957 | 2.3064 |
| 10.63 | 1.264 | 9.9331 | 2639.8543 | 2.2767 |
| 10.64 | 1.255 | 9.9337 | 2640.0043 | 2.2471 |
| 10.64 | 1.246 | 9.9583 | 2646.5448 | 2.2174 |
| 10.65 | 1.238 | 9.8870 | 2627.6106 | 2.1881 |
| 10.65 | 1.230 | 9.8034 | 2605.3981 | 2.1591 |
| 10.66 | 1.221 | 9.6954 | 2576.6715 | 2.1305 |
| 10.66 | 1.213 | 9.6594 | 2567.1174 | 2.1021 |
| 10.67 | 1.205 | 9.6840 | 2573.6657 | 2.0736 |
| 10.67 | 1.197 | 9.7454 | 2589.9787 | 2.0451 |
| 10.68 | 1.189 | 9.7706 | 2596.6726 | 2.0166 |
| 10.68 | 1.181 | 9.7593 | 2593.6677 | 1.9882 |
| 10.69 | 1.173 | 9.7849 | 2600.4562 | 1.9598 |
| 10.69 | 1.165 | 9.7368 | 2587.6779 | 1.9316 |
| 10.70 | 1.157 | 9.6021 | 2551.8860 | 1.9039 |
| 10.70 | 1.150 | 9.5410 | 2535.6540 | 1.8764 |
| 10.71 | 1.142 | 9.5293 | 2532.5503 | 1.8491 |
| 10.71 | 1.134 | 9.6297 | 2559.2330 | 1.8215 |
| 10.72 | 1.127 | 9.6559 | 2566.1823 | 1.7940 |
| 10.72 | 1.120 | 9.6823 | 2573.1957 | 1.7664 |
| 10.73 | 1.112 | 9.7089 | 2580.2742 | 1.7389 |
| 10.73 | 1.105 | 9.7358 | 2587.4184 | 1.7114 |
| 10.74 | 1.098 | 9.7250 | 2584.5555 | 1.6839 |
| 10.74 | 1.091 | 9.6382 | 2561.4920 | 1.6569 |
| 10.75 | 1.084 | 9.5383 | 2534.9359 | 1.6301 |
| 10.75 | 1.077 | 9.5781 | 2545.5143 | 1.6033 |
| 10.76 | 1.070 | 9.6310 | 2559.5798 | 1.5765 |
| 10.76 | 1.063 | 9.6587 | 2566.9375 | 1.5497 |
| 10.77 | 1.056 | 9.7253 | 2584.6236 | 1.5228 |
| 10.77 | 1.050 | 9.7536 | 2592.1534 | 1.4958 |
| 10.78 | 1.043 | 9.7434 | 2589.4323 | 1.4690 |
| 10.78 | 1.036 | 9.7461 | 2590.1706 | 1.4423 |
| 10.79 | 1.030 | 9.6839 | 2573.6253 | 1.4158 |
| 10.79 | 1.023 | 9.5691 | 2543.1050 | 1.3897 |
| 10.80 | 1.017 | 9.5585 | 2540.2924 | 1.3638 |
| 10.80 | 1.011 | 9.5611 | 2540.9824 | 1.3379 |
| 10.81 | 1.004 | 9.5638 | 2541.7015 | 1.3120 |
| 10.81 | 998 | 9.5136 | 2528.3751 | 1.2864 |
| 10.82 | 992 | 9.4632 | 2514.9861 | 1.2611 |
| 10.82 | 986 | 9.4393 | 2508.6174 | 1.2358 |
| 10.83 | 980 | 9.3484 | 2484.4624 | 1.2109 |
| 10.83 | 974 | 9.2033 | 2445.9079 | 1.1865 |
| 10.84 | 968 | 9.0304 | 2399.9632 | 1.1626 |
| 10.84 | 962 | 8.8835 | 2360.9110 | 1.1391 |
| 10.85 | 956 | 8.8441 | 2350.4328 | 1.1159 |
| 10.85 | 951 | 8.7637 | 2329.0632 | 1.0929 |
| 10.86 | 945 | 8.7101 | 2314.8234 | 1.0702 |
| 10.86 | 939 | 8.5330 | 2267.7529 | 1.0480 |
| 10.87 | 934 | 8.4234 | 2238.6375 | 1.0262 |
| 10.87 | 928 | 8.2718 | 2198.3451 | 1.0048 |
| 10.88 | 923 | 8.0915 | 2150.4340 | 0.9840 |
| 10.88 | 917 | 7.8684 | 2091.1301 | 0.9638 |
| 10.89 | 912 | 7.7970 | 2072.1713 | 0.9438 |
| 10.89 | 906 | 7.6833 | 2041.9436 | 0.9243 |
| 10.90 | 901 | 7.5548 | 2007.7864 | 0.9051 |
| 10.90 | 896 | 7.4254 | 1973.4003 | 0.8863 |
| 10.91 | 891 | 7.2810 | 1935.0232 | 0.8680 |

MW = 1000 max



| | | | | |
|-------|-----|--------|-----------|--------|
| 10.91 | 885 | 7.1924 | 1911.4729 | 0.8499 |
| 10.92 | 880 | 7.1459 | 1899.1275 | 0.8321 |
| 10.92 | 875 | 7.0563 | 1875.3066 | 0.8145 |
| 10.93 | 870 | 6.9374 | 1843.6949 | 0.7973 |
| 10.93 | 865 | 6.8464 | 1819.5222 | 0.7803 |
| 10.94 | 860 | 6.8127 | 1810.5623 | 0.7636 |
| 10.94 | 856 | 6.7497 | 1793.8339 | 0.7470 |
| 10.95 | 851 | 6.6718 | 1773.1208 | 0.7307 |
| 10.95 | 846 | 6.5933 | 1752.2651 | 0.7147 |
| 10.96 | 841 | 6.6172 | 1758.5982 | 0.6986 |
| 10.96 | 837 | 6.5675 | 1745.3992 | 0.6827 |
| 10.97 | 832 | 6.5916 | 1751.7937 | 0.6669 |
| 10.97 | 827 | 6.6754 | 1774.0656 | 0.6509 |
| 10.98 | 823 | 6.6703 | 1772.7307 | 0.6350 |
| 10.98 | 818 | 6.6204 | 1759.4529 | 0.6192 |
| 10.99 | 814 | 6.6002 | 1754.0864 | 0.6036 |
| 10.99 | 809 | 6.5648 | 1744.6785 | 0.5881 |
| 11.00 | 805 | 6.5899 | 1751.3456 | 0.5727 |
| 11.00 | 801 | 6.6152 | 1758.0881 | 0.5572 |
| 11.01 | 796 | 6.5644 | 1744.5707 | 0.5419 |
| 11.01 | 792 | 6.6361 | 1763.6349 | 0.5265 |
| 11.02 | 788 | 6.7548 | 1795.1877 | 0.5109 |
| 11.02 | 784 | 6.7196 | 1785.8358 | 0.4955 |
| 11.03 | 780 | 6.6998 | 1780.5610 | 0.4802 |
| 11.03 | 776 | 6.6486 | 1766.9498 | 0.4650 |
| 11.04 | 772 | 6.6441 | 1765.7610 | 0.4499 |
| 11.04 | 768 | 6.6239 | 1760.3900 | 0.4350 |
| 11.05 | 764 | 6.5561 | 1742.3592 | 0.4202 |
| 11.05 | 760 | 6.4877 | 1724.1873 | 0.4057 |
| 11.06 | 756 | 6.6106 | 1756.8582 | 0.3910 |
| 11.06 | 752 | 6.6866 | 1777.0422 | 0.3762 |
| 11.07 | 748 | 6.6342 | 1763.1301 | 0.3615 |
| 11.07 | 744 | 6.5004 | 1727.5730 | 0.3472 |
| 11.08 | 741 | 6.4469 | 1713.3443 | 0.3331 |
| 11.08 | 737 | 6.3438 | 1685.9597 | 0.3193 |
| 11.09 | 733 | 6.2399 | 1658.3375 | 0.3058 |
| 11.09 | 729 | 6.0525 | 1608.5278 | 0.2927 |
| 11.10 | 726 | 6.0293 | 1602.3627 | 0.2798 |
| 11.10 | 722 | 6.0893 | 1618.3150 | 0.2668 |
| 11.11 | 719 | 6.0830 | 1616.6303 | 0.2538 |
| 11.11 | 715 | 5.9083 | 1570.2022 | 0.2413 |
| 11.12 | 712 | 5.6981 | 1514.3428 | 0.2293 |
| 11.12 | 708 | 5.5709 | 1480.5437 | 0.2176 |
| 11.13 | 705 | 5.3913 | 1432.8001 | 0.2064 |
| 11.13 | 702 | 5.1927 | 1380.0290 | 0.1956 |
| 11.14 | 698 | 5.0785 | 1349.6791 | 0.1852 |
| 11.14 | 695 | 4.9805 | 1323.6474 | 0.1749 |
| 11.15 | 692 | 4.9863 | 1325.1751 | 0.1647 |
| 11.15 | 688 | 4.9045 | 1303.4397 | 0.1548 |
| 11.16 | 685 | 4.7514 | 1262.7593 | 0.1452 |
| 11.16 | 682 | 4.5260 | 1202.8327 | 0.1361 |
| 11.17 | 679 | 4.2625 | 1132.8181 | 0.1275 |
| 11.17 | 676 | 4.0500 | 1076.3448 | 0.1195 |
| 11.18 | 673 | 3.8533 | 1024.0605 | 0.1119 |
| 11.18 | 670 | 3.7450 | 995.2738 | 0.1045 |
| 11.19 | 667 | 3.6537 | 971.0217 | 0.0973 |
| 11.19 | 664 | 3.6164 | 961.1060 | 0.0903 |
| 11.20 | 661 | 3.5051 | 931.5290 | 0.0835 |
| 11.20 | 658 | 3.4111 | 906.5508 | 0.0769 |
| 11.21 | 655 | 3.1300 | 831.8386 | 0.0710 |
| 11.21 | 652 | 2.8644 | 761.2593 | 0.0655 |
| 11.22 | 649 | 2.6900 | 714.8944 | 0.0604 |
| 11.22 | 646 | 2.5514 | 678.0660 | 0.0556 |
| 11.23 | 644 | 2.4493 | 650.9397 | 0.0510 |
| 11.23 | 641 | 2.4036 | 638.7779 | 0.0466 |
| 11.24 | 638 | 2.3380 | 621.3582 | 0.0422 |
| 11.24 | 635 | 2.3105 | 614.0442 | 0.0380 |
| 11.25 | 633 | 2.2242 | 591.0981 | 0.0339 |
| 11.25 | 630 | 2.0190 | 536.5859 | 0.0303 |
| 11.26 | 627 | 1.7522 | 465.6666 | 0.0271 |

| 11.26 | 625 | 1.5814 | 420.2763 | 0.0243 |
|--|-----|-----------|-------------|----------|
| 11.27 | 622 | 1.4685 | 390.2648 | 0.0216 |
| 11.27 | 620 | 1.3139 | 349.1854 | 0.0193 |
| 11.28 | 617 | 1.3194 | 350.6386 | 0.0170 |
| 11.28 | 615 | 1.2842 | 341.2838 | 0.0148 |
| 11.29 | 612 | 1.2895 | 342.7151 | 0.0125 |
| 11.29 | 610 | 1.1918 | 316.7361 | 0.0105 |
| 11.30 | 607 | 1.0512 | 279.3728 | 0.0087 |
| 11.30 | 605 | 0.8460 | 224.8364 | 0.0072 |
| 11.31 | 603 | 0.7223 | 191.9598 | 0.0060 |
| 11.31 | 600 | 0.5333 | 141.7342 | 0.0051 |
| 11.32 | 598 | 0.4912 | 130.5411 | 0.0043 |
| 11.32 | 596 | 0.5345 | 142.0483 | 0.0034 |
| 11.33 | 593 | 0.5568 | 147.9650 | 0.0025 |
| 11.33 | 591 | 0.5357 | 142.3803 | 0.0016 |
| 11.34 | 589 | 0.4925 | 130.8837 | 0.0008 |
| 11.34 | 587 | 0.3602 | 95.7177 | 0.0002 |
| 11.35 | 585 | 0.1368 | 36.3671 | 0.0000 |
| 11.35 | 582 | 0.0000 | 0.0000 | 0.0000 |
| ピークNo : 2 時間[分] : 11.44 ~ 11.63 ~ 11.88 | | | | |
| 時間[分] | 分子量 | 微分分布[面積] | 微分分布[高さ] | 積分分布 |
| 11.44 | 549 | 0.0000 | 0.0000 | 100.0000 |
| 11.44 | 548 | 8.3020 | 341.3694 | 99.9886 |
| 11.45 | 546 | 12.7711 | 525.1338 | 99.9712 |
| 11.45 | 544 | 39.1880 | 1611.3621 | 99.9183 |
| 11.46 | 543 | 66.0856 | 2717.3613 | 99.8299 |
| 11.46 | 541 | 85.8950 | 3531.8976 | 99.7160 |
| 11.47 | 539 | 113.7288 | 4676.3929 | 99.5666 |
| 11.47 | 538 | 147.2395 | 6054.3112 | 99.3750 |
| 11.48 | 536 | 173.5905 | 7137.8345 | 99.1512 |
| 11.48 | 534 | 208.3278 | 8566.1869 | 98.8853 |
| 11.49 | 533 | 238.4445 | 9804.5528 | 98.5838 |
| 11.49 | 531 | 298.6055 | 12278.3001 | 98.2099 |
| 11.50 | 530 | 351.8624 | 14468.1597 | 97.7737 |
| 11.50 | 528 | 408.9334 | 16814.8509 | 97.2718 |
| 11.51 | 527 | 480.9746 | 19777.0978 | 96.6875 |
| 11.51 | 525 | 548.9429 | 22571.8695 | 96.0275 |
| 11.52 | 524 | 618.3481 | 25425.7302 | 95.2918 |
| 11.52 | 523 | 706.3207 | 29043.0555 | 94.4602 |
| 11.53 | 521 | 787.5692 | 32383.8959 | 93.5428 |
| 11.53 | 520 | 882.2403 | 36276.6601 | 92.5262 |
| 11.54 | 518 | 981.9622 | 40377.1053 | 91.4071 |
| 11.54 | 517 | 1080.9637 | 44447.9272 | 90.1888 |
| 11.55 | 516 | 1176.2198 | 48364.7447 | 88.8780 |
| 11.55 | 514 | 1298.0289 | 53373.3870 | 87.4479 |
| 11.56 | 513 | 1413.4739 | 58120.3462 | 85.9086 |
| 11.56 | 512 | 1519.2242 | 62468.6710 | 84.2734 |
| 11.57 | 511 | 1630.6958 | 67052.2476 | 82.5390 |
| 11.57 | 509 | 1751.2947 | 72011.1314 | 80.6988 |
| 11.58 | 508 | 1861.9941 | 76562.9573 | 78.7660 |
| 11.58 | 507 | 1955.8931 | 80423.9719 | 76.7609 |
| 11.59 | 506 | 2055.5358 | 84521.1571 | 74.6800 |
| 11.59 | 504 | 2157.7981 | 88726.0593 | 72.5234 |
| 11.60 | 503 | 2242.4070 | 92205.0769 | 70.3112 |
| 11.60 | 502 | 2325.8795 | 95637.3661 | 68.0468 |
| 11.61 | 501 | 2411.6654 | 99164.7792 | 65.7300 |
| 11.61 | 500 | 2485.7201 | 102209.8183 | 63.3744 |
| 11.62 | 499 | 2569.0762 | 105637.3229 | 60.9732 |
| 11.62 | 498 | 2633.0419 | 108267.5149 | 58.5466 |
| 11.63 | 497 | 2676.7879 | 110066.2982 | 56.1146 |
| 11.63 | 496 | 2706.9188 | 111305.2454 | 53.6907 |
| 11.64 | 495 | 2734.2269 | 112428.1178 | 51.2782 |
| 11.64 | 494 | 2743.1629 | 112795.5591 | 48.8939 |
| 11.65 | 493 | 2752.4579 | 113177.7581 | 46.5377 |
| 11.65 | 492 | 2754.1765 | 113248.4216 | 44.2164 |
| 11.66 | 491 | 2743.9172 | 112826.5744 | 41.9400 |
| 11.66 | 490 | 2753.9411 | 113238.7426 | 39.6918 |
| 11.67 | 489 | 2760.2128 | 113496.6269 | 37.4751 |
| 11.67 | 488 | 2711.6204 | 111498.5670 | 35.3335 |

| | | | | |
|-------|-----|-----------|-------------|---------|
| 11.68 | 487 | 2674.4028 | 109968.2234 | 33.2570 |
| 11.68 | 486 | 2622.8001 | 107846.3853 | 31.2555 |
| 11.69 | 486 | 2569.4495 | 105652.6719 | 29.3291 |
| 11.69 | 485 | 2523.3534 | 103757.2546 | 27.4711 |
| 11.70 | 484 | 2466.3650 | 101413.9626 | 25.6882 |
| 11.70 | 483 | 2430.9294 | 99956.8904 | 23.9636 |
| 11.71 | 482 | 2384.5821 | 98051.1447 | 22.3041 |
| 11.71 | 482 | 2351.2267 | 96679.6115 | 20.6995 |
| 11.72 | 481 | 2296.5728 | 94432.3084 | 19.1633 |
| 11.72 | 480 | 2224.4081 | 91464.9849 | 17.7056 |
| 11.73 | 479 | 2128.3911 | 87516.8789 | 16.3397 |
| 11.73 | 479 | 2065.6546 | 84937.2327 | 15.0422 |
| 11.74 | 478 | 1967.4984 | 80901.1664 | 13.8332 |
| 11.74 | 477 | 1920.7494 | 78978.9032 | 12.6792 |
| 11.75 | 477 | 1854.7824 | 76266.4199 | 11.5902 |
| 11.75 | 476 | 1826.6614 | 75110.1184 | 10.5428 |
| 11.76 | 475 | 1797.2460 | 73900.5926 | 9.5369 |
| 11.76 | 475 | 1748.0151 | 71876.2778 | 8.5827 |
| 11.77 | 474 | 1633.3302 | 67160.5717 | 7.7137 |
| 11.77 | 474 | 1532.0156 | 62994.6383 | 6.9197 |
| 11.78 | 473 | 1465.0887 | 60242.6851 | 6.1807 |
| 11.78 | 472 | 1401.2893 | 57619.3287 | 5.4933 |
| 11.79 | 472 | 1298.6886 | 53400.5103 | 4.8743 |
| 11.79 | 471 | 1262.4252 | 51909.4031 | 4.2902 |
| 11.80 | 471 | 1253.8577 | 51557.1176 | 3.7276 |
| 11.80 | 470 | 1267.9428 | 52136.2839 | 3.1764 |
| 11.81 | 470 | 1195.4197 | 49154.2193 | 2.6734 |
| 11.81 | 470 | 1052.0460 | 43258.8665 | 2.2455 |
| 11.82 | 469 | 949.6980 | 39050.4391 | 1.8726 |
| 11.82 | 469 | 839.8046 | 34531.7525 | 1.5547 |
| 11.83 | 468 | 758.2531 | 31178.4547 | 1.2784 |
| 11.83 | 468 | 660.6122 | 27163.5779 | 1.0470 |
| 11.84 | 468 | 654.5108 | 26912.6958 | 0.8270 |
| 11.84 | 467 | 647.9253 | 26641.9084 | 0.6184 |
| 11.85 | 467 | 629.8951 | 25900.5295 | 0.4246 |
| 11.85 | 467 | 564.4896 | 23211.1347 | 0.2589 |
| 11.86 | 466 | 432.4333 | 17781.1364 | 0.1382 |
| 11.86 | 466 | 324.1333 | 13327.9718 | 0.0523 |
| 11.87 | 466 | 136.6152 | 5617.4510 | 0.0181 |
| 11.87 | 465 | 11.4785 | 471.9808 | 0.0154 |
| 11.88 | 465 | 69.4303 | 2854.8895 | 0.0000 |
| 11.88 | 465 | 0.0000 | 0.0000 | 0.0000 |

ACUTE TOXICITY TO *DAPHNIA MAGNA*

HLS study number:

Version ID:

Final

Issue date:

19 August 2008

Sponsor and Test Facility Details

Sponsor

Test Facility

Huntingdon Life Sciences
Eye Research Centre
Eye
Suffolk
IP23 7PX
UK

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Compliance with Good Laboratory Practice

Acute Toxicity to *Daphnia magna*


The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

These principles of Good Laboratory Practice are accepted by the Regulatory Authorities of the United States of America and Japan on the basis of Intergovernmental Agreements.



Jennifer J Wilby BSc MIBiol
Study Director
Huntingdon Life Sciences Ltd

19 August 2008

Date

Quality Assurance Statement

Acute Toxicity to *Daphnia magna*

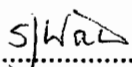
The following inspections and audits have been carried out in relation to this study:

| Study Phase | Date(s) of Inspection | Date of Reporting to Study Director and Management |
|----------------|-----------------------|--|
| Protocol Audit | 17 March 2008 | 17 March 2008 |
| Report Audit | 24 June 2008 | 24 June 2008 |

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

| Process Based Inspections | Date(s) of Inspection | Date of Reporting to Management |
|--|-----------------------|---------------------------------|
| Dose Formulation | 3 June 2008 | 3 June 2008 |
| Experimental Set-up | 10 April 2008 | 10 April 2008 |
| Observations | 11 April 2008 | 11 April 2008 |
| Sampling of Test Media for Chemical Analysis | 3 June 2008 | 3 June 2008 |
| Carbon Analysis | 27 February 2008 | 27 February 2008 |

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.



 Sarah J Watts CBiol MIBiol MRQA
 Principal Auditor
 Department of Quality Assurance
 Huntingdon Life Sciences Ltd.

19 August 2008

 Date

Contributing Scientists

Acute Toxicity to *Daphnia magna*

Study management

Jennifer J Wilby BSc MIBiol
Study Director (replacement)
Aquatic Ecotoxicology and Biodegradation

Kim Utting Cert NatSci (Open)
Study Director (original)
Aquatic Ecotoxicology and Biodegradation

Jonathan Burke BSc MRes
Study Manager
Aquatic Ecotoxicology and Biodegradation

Robert A Dickinson BSc
Study Manager
Aquatic Ecotoxicology and Biodegradation (Carbon Analysis)

Georgina L Podd
Laboratory Technician
Aquatic Ecotoxicology and Biodegradation (Carbon Analysis)

Summary

The acute toxicity of _____ (tested as a WAF; water accommodated fraction) to *Daphnia magna* was assessed under static exposure conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity to *Daphnia*" and the OECD Guideline for Testing of Chemicals No. 202, "*Daphnia* Acute Immobilisation Test" (2004).

Groups of twenty *Daphnia*, less than 24 hours old, were exposed for 48 hours to a WAF prepared from an aqueous mixture with an initial nominal concentration of 10 g/l. The test media was prepared in Elendt M4 medium by the direct addition of the test substance to the dilution medium. To aid dissolution, the test mixture was stirred overnight in the dark before being left to stand for approximately 3 hours. The aqueous phase (WAF, approximately 700 ml) was then removed and used as the test medium.

The composition of the test substance made it unsuitable for analysis using chromatographic methods. Consequently, the exposure level was monitored using Total and Dissolved Organic Carbon (TOC and DOC). At the start of the test, the mean measured levels of TOC and DOC in samples of the control and test medium were 1.15 and 0.1 mg C/l respectively. After 48 hours, the mean measured levels of TOC in samples of the control and test medium were 1.20 and 0.2 mg C/l respectively and levels of DOC were 1.20 and 0 mg C/l. Results of a water extractability test conducted using a pH 7 buffered solution of _____ at a loading rate of 10 g/l indicated a carbon content of 0.18 mg C/l. The results achieved in the test confirm the low solubility of the test substance.

Observations of the *Daphnia* in each control and test vessel were made after approximately 24 and 48 hours. No immobilisation or adverse effects on the *Daphnia* were observed.

Based on these findings the following values have been estimated:

48-hour EC₅₀ value : >10 g/l*
"No observed effect concentration": 10 g/l*

* : nominal concentration of the initial aqueous mixture used to prepare the WAF.

1. Introduction

The objective of the study was to determine the acute toxicity (48 hour median effect concentration - EC₅₀) of _____ to *Daphnia magna*.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity to *Daphnia*" and the OECD Guideline for Testing of Chemicals No. 202, "*Daphnia* Acute Immobilisation Test" (2004). The design of the study was in accordance with recommendations received from the UK Environment Agency (May 2007), which proposed the use of a WAF.

The protocol was approved by Huntingdon Life Sciences Management and the Study Director on 7 March 2008 and by the Sponsor on 23 January 2008.

The experimental start and completion dates of the study were 17 March and 26 March 2008, respectively.

The study was conducted at Huntingdon Life Sciences Ltd, Eye Research Centre, Eye, Suffolk, IP23 7PX, England.

The composition of the test material made it unsuitable for analysis using chromatographic methods. Consequently, Total and Dissolved Organic Carbon analysis was undertaken on samples of media taken at the start and end of the test.

Information provided by the Sponsor indicated that _____ was insoluble in water. Results of a water extractability test conducted using a pH 7 buffered solution of _____ at a loading rate of 10 g/l indicated a carbon content of 0.18 mg C/l.

In accordance with the recommendation of the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (Number 23), the test results have been expressed in terms of the nominal loading rate (i.e. the nominal weight of the test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was removed).

2. Test substance

Identity:

Chemical name:

Lot number:

Expiry Date: 12 December 2008

Appearance: White powder

Storage conditions: Room temperature in the dark, desiccated

Purity/assay: 99.55%

Water solubility: Insoluble

Sample received (Huntingdon Site): 10 January 2008

The Sponsor's Certificate of analysis is given in Appendix 1

3. Experimental procedure

3.1 Test Organism

Daphnia magna (Straus) used in this study were cultured in-house and were obtained from a strain originating from the National Institute for Applied Chemical Research (IRCHA), France.

Stock cultures of *Daphnia magna* were maintained in glass vessels containing approximately 0.5 to 0.8 litres of Elendt M4 culture medium in a temperature-controlled laboratory at nominally $20 \pm 2^\circ\text{C}$. A photoperiod of 16 hours light : 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The culture medium was renewed three times each week.

Cultures were fed daily with a suspension of the unicellular green algae, *Pseudokirchneriella subcapitata*, to provide nominally 0.1 to 0.2 mg carbon per daphnid, per day, except during the initial three days when a slightly lower ration was given. Culture conditions ensure that the stock animals reproduce by parthenogenesis.

The day before the start of the study, all juvenile *Daphnia* were removed from the laboratory cultures. The following morning, juveniles produced by the gravid (egg-bearing) adult *Daphnia* were removed from the culture vessels and held in a separate holding vessel; these animals, which were less than 24 hours old, were used in the test.

3.2 Dilution Medium

The test organisms were maintained and the tests conducted in Elendt M4 medium (Appendix 2). The medium was prepared in deionised, reverse osmosis water.

3.3 Test substance preparation

The method of preparation used during the test was based on recommendations by the UK Environment Agency Chemicals Assessment Unit (May 2007).

The test substance (10 g) was dispersed in culture medium (1 l) in a glass vessel. This aqueous mixture was stirred overnight in the dark before being left to stand for approximately 3 hours. An aliquot (700 ml) of the aqueous phase (WAF) was then removed from mid position from the preparation vessel and was used as the test medium.

3.4 Exposure Conditions

The study comprised a single definitive (limit) test.

3.4.1 Experimental design

Twenty *Daphnia*, four replicates of five animals per vessel, were exposed in each control and test group.

The first instar *Daphnia* were placed in groups of five, at random into glass dishes containing 100 ml of medium to give a loading of 20 ml medium per organism. The dishes were loosely covered.

3.4.2 Test concentrations

The test employed a nominal loading rate of 10 g/l. This concentration was based on the nominal weight of test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was prepared.

3.4.3 Medium renewal

Daphnia were exposed to the test or control conditions for a period of 48 hours without renewal of test media.

3.4.4 Stability of test concentrations

Total and Dissolved Organic Carbon (TOC and DOC) analysis (Appendix 3) was undertaken on samples of media taken at the start and end of the test.

At the start of the test, four samples (50 ml) were taken from the freshly-prepared control and test media; after 48 hours, the contents of the replicate vessels for each group were pooled and eight further samples were taken for analysis. All samples were stored in a freezer until defrosted for analysis when required.

Two samples for each sampling point were analysed for Total Organic Carbon and two were filtered (0.45µm cellulose nitrate) before analysis for Dissolved Organic Carbon.

3.4.5 Environmental conditions

The temperature of the test area was maintained at $20 \pm 2^{\circ}\text{C}$ during the test. Temperature was continuously monitored in an additional vessel containing the same volume of dilution medium. A photoperiod of 16 hours light : 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. No supplementary aeration was employed and no feed was given during the exposure period.

The temperature, pH and dissolved oxygen levels of control and test media were recorded at the start and at the end of the study. The total hardness and alkalinity of the dilution medium was measured before use.

3.5 Criterion of effect

Daphnia were considered to be immobile if they were unable to swim within approximately 15 seconds following gentle agitation of the test vessel.

The numbers of mobile, immobile and floating *Daphnia* were counted approximately 24 and 48 hours after the start of the study.

3.6 Evaluation of data

The “no observed effect concentration” (NOEC) was derived by direct inspection of the data on the immobility of the animals. An incidence rate of more than 10% is considered to be significant.

3.7 Protocol Deviations

None.

4. Maintenance of records

All specimens (if appropriate), raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Archive.

Specimens and records will be retained for a minimum period of one year from the date of issue of the final report. At the end of the one year retention period the Sponsor will be contacted and advice sought on their future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

5. Results

5.1 Chemical analysis

The results of carbon analysis are given in Table 1.

At the start of the test, the mean measured (blank corrected) TOC and DOC levels in samples of the control and test media were 1.15 and 0.1 mg C/l respectively.

After 48 hours, the mean measured (blank corrected) levels of TOC in samples of the control and test media were 1.20 and 0.2 mg C/l respectively; the DOC levels were 1.20 and 0 mg C/l.

In accordance with the recommendation of the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (Number 23), the test results have been expressed in terms of the nominal loading rate (i.e. the nominal weight of the test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was removed).

5.2 Immobility

Observations of the *Daphnia* in each control and test vessel made after 24 and 48 hours. No immobilisation or adverse effects on the *Daphnia* were noted.

Based on these findings the following values have been estimated:

| | | |
|-------------------------------------|---|----------|
| 48-hour EC ₅₀ value | : | >10 g/l* |
| "No observed effect concentration": | | 10 g/l* |

* : nominal concentration of the initial aqueous mixture used to prepare the WAF.

5.3 Environmental parameters

The measurements of water quality (temperature, pH, dissolved oxygen, total hardness and alkalinity) are summarised in Table 3; they remained within acceptable limits during the study.

The test medium was a non-homogeneous, hazy dispersion.

6. Conclusions

Under the conditions of the study, a WAF of the _____ was not found to be acutely toxic to *Daphnia magna* at a nominal loading rate of 10 g/l.

Consequently, the 48-hour EC₅₀ value for _____ with *Daphnia magna* could not be calculated but must be >10 g/l and the “no observed effect concentration” was 10 g/l.

7. References

UK ENVIRONMENT AGENCY CHEMICALS ASSESSMENT UNIT
RECOMMENDATIONS (2007). Notification of New Substances Regs. (1993): Level 1B
Testing Proposal

OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures
(Number 23) ENV/JM/MONO(2000)6

Table 1 Measured TOC and DOC concentrations

| Nominal Loading Rate (g/l) | Measured TOC concentrations (mg C/l) | | | |
|----------------------------------|--------------------------------------|---------------|----------|---------------|
| | 0 hours | | 48 hours | |
| Control | 0.9 | 1.4 (1.15) | 1.2 | 1.2 (1.20) |
| 10* | 0.05 | 0.15 (0.1) | 0.0 | 0.4 (0.2) |

* : blank corrected values (measured concentration - mean control value for sampling occasion)
 () : mean measured concentration

| Nominal Loading Rate (g/l) | Measured DOC concentrations (mg C/l) | | | |
|----------------------------------|--------------------------------------|---------------|----------|---------------|
| | 0 hours | | 48 hours | |
| Control | 1.1 | 1.2 (1.15) | 1.2 | 1.2 (1.20) |
| 10* | 0.15 | 0.05 (0.1) | 0.0 | 0.0 (0.0) |

* : blank corrected values (measured concentration - mean control value for sampling occasion)
 () : mean measured concentration

Table 2 Environmental parameters

| Nominal Loading Rate (g/l) | Temperature (°C) | | pH | | Dissolved oxygen (%ASV) | |
|-------------------------------|------------------|------|------|------|----------------------------|------|
| | 0 h | 48 h | 0 h | 48 h | 0 h | 48 h |
| Control | 19.1 | 20.1 | 7.42 | 7.49 | 90 | 89 |
| 10 | 19.6 | 20.0 | 7.55 | 7.53 | 92 | 90 |

ASV : air saturation value.

The total hardness of the batches of Elendt M4 medium used in the study were 240 and 248 mg CaCO₃. The measured alkalinity was 50.1 mg/l as CaCO₃.

Continuous monitoring of an additional vessel containing dilution medium = 19.0 to 20.2°C.

Appendix 1 Certificate of Analysis**CERTIFICATE OF ANALYSIS**

NAME OF SAMPLE

LOT NO. OF SAMPLE :

DATE OF ANALYSIS : 12 December 2008

COMPOSITION

| Identity | Cas No. | Conc. |
|--------------|---------|-------------|
| #1 | | |
| #2 | | |
| #3 | | |
| #4 | | % |
| #5 | | % |
| TOTAL | | 100% |

NAME :

SIGNATUREDATE 23 June 2008

Appendix 2 Elendt M4 Medium

| | | |
|----|--|---------|
| 1. | Micro elements | mg/l |
| | H ₃ BO ₃ | 2.86 |
| | MnCl ₂ .4H ₂ O | 0.36 |
| | LiCl | 0.31 |
| | RbCl | 0.071 |
| | SrCl ₂ .6H ₂ O | 0.152 |
| | NaBr | 0.016 |
| | Na ₂ MoO ₄ .2H ₂ O | 0.063 |
| | CuCl ₂ .2H ₂ O | 0.017 |
| | ZnCl ₂ | 0.013 |
| | CoCl ₂ .6H ₂ O | 0.010 |
| | KI | 0.0033 |
| | Na ₂ .SeO ₃ | 0.0022 |
| | NH ₄ VO ₃ | 0.00058 |
| | Fe-EDTA solution | 3.50 |
| 2. | Macro nutrients | mg/l |
| | CaCl ₂ .2H ₂ O | 294 |
| | MgSO ₄ .7H ₂ O | 123 |
| | KCl | 5.80 |
| | NaHCO ₃ | 64.8 |
| 3. | Buffer nutrients | mg/l |
| | Na ₂ .SiO ₃ .9H ₂ O | 10 |
| | NaNO ₃ | 0.274 |
| | KH ₂ PO ₄ | 0.143 |
| | K ₂ HPO ₄ | 0.184 |
| 4. | Vitamins | mg/l |
| | Thiamine hydrochloride | 0.075 |
| | Cyanocobalamine (B12) | 0.0010 |
| | Biotin | 0.00075 |

The above analytical grade reagents are dissolved in deionised water produced by reverse osmosis.

Appendix 3 Total and Dissolved Organic Carbon Analysis (TOC and DOC)

Introduction

TOC or DOC concentrations in aqueous mixtures were determined using an OI Model 700 carbon analyser. Samples for DOC analysis were prepared by filtration of the medium (0.45 µm membrane filters).

Organic carbon concentrations were measured in this analysis by the amount of carbon dioxide released by chemical oxidation of the aqueous sample at 100°C in the presence of phosphoric acid and sodium persulphate. The carbon dioxide was purged from the sample, concentrated by trapping and desorbed and carried to a non-dispersive infrared detector (NDIR) whose output was calibrated (using aqueous solutions of potassium hydrogen phthalate) to directly display the concentration of carbon present in the sample. Sample volumes were measured using a calibrated sample loop (1.02 ml) and phosphoric acid then sodium persulphate were automatically injected with each sample.

Materials

Chemicals used in carbon analysis

Potassium hydrogen phthalate (Analar grade) used as a standard.

Sodium persulphate (98+%).

Phosphoric acid (85% Orthophosphoric acid, analytical grade; SG 1.7) for acidification of samples as part of the analysis in "TIC/TOC" mode.

Dilution water

The dilution water used to prepare solutions was tap water that had been softened and treated by reverse osmosis then purified; resistivity $\geq 18 \text{ M}\Omega\cdot\text{cm}$. This water complied with the relevant British Standard and American Society for Testing and Materials for classification as Grade 1 or Type 1 Laboratory Water, respectively.

Determination of carbon levels

The carbon analyser was calibrated using at least five samples of an aqueous solution of potassium hydrogen phthalate (20 mg C/l). At least five samples of ultrapure water verified the absence of contamination within the carbon analyser and the TOC content of five samples of the reagents (sodium persulphate and phosphoric acid) were analysed to obtain a mean blank value in the calibration. These reagent blanks were entered into the calculation program of the analyser. The potassium hydrogen phthalate solution was analysed at the end of each set of analyses and verified the stability of the detector response.

The TOC/DOC content of dilution medium control, solvent control and test medium was determined in the TIC/TOC mode of analysis. Samples injected into the analyser were acidified (phosphoric acid; 0.4 ml of a 5% v/v solution) then purged with nitrogen in order to release inorganic carbon. The oxidant (sodium persulphate; 1.0 ml of a 100 g/l solution) was automatically added to the sample and the mixture heated to approximately 100°C in the analyser to oxidise the organic carbon to carbon dioxide which was quantified.

Appendix 4 Eye Research Centre GLP Compliance Statement 2008



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

28th January 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

4/03/08

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



ALGAL GROWTH INHIBITION ASSAY

HLS study number:

Version ID:

Final

Issue date:

19 August 2008

Sponsor and Test Facility Details

Sponsor

Test Facility

Huntingdon Life Sciences
Eye Research Centre
Eye
Suffolk
IP23 7PX
UK

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Compliance with Good Laboratory Practice

Algal growth inhibition assay

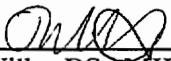
The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

These principles of Good Laboratory Practice are accepted by the Regulatory Authorities of the United States of America and Japan on the basis of Intergovernmental Agreements.



Jennifer J Wilby BSc MIBiol
Study Director
Huntingdon Life Sciences Ltd

19 August 2008

Date

Quality Assurance Statement

Algal growth inhibition assay

The following inspections and audits have been carried out in relation to this study:

| Study Phase | Date(s) of Inspection | Date of Reporting to Study Director and Management |
|----------------|-----------------------|--|
| Protocol Audit | 17 March 2008 | 17 March 2008 |
| Report Audit | 24-25 June 2008 | 25 June 2008 |

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

| Process Based Inspections | Date(s) of Inspection | Date of Reporting to Management |
|--|-----------------------|---------------------------------|
| Dose Formulation | 3 June 2008 | 3 June 2008 |
| Experimental Set-up | 14 April 2008 | 14 April 2008 |
| Counting of Cells | 30 May 2008 | 30 May 2008 |
| Sampling of Test Media for Chemical Analysis | 3 June 2008 | 3 June 2008 |
| Carbon Analysis | 27 February 2008 | 27 February 2008 |

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.

.....*SJW*.....

Sarah J Watts CBiol MIBiol MRQA
Principal Auditor
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

.....19 August 2008.....

Date

Contributing Scientists

Algal growth inhibition assay

Study management

Jennifer J. Wilby BSc MIBiol
Study Director (replacement)
Aquatic Ecotoxicology and Biodegradation

Kim Utting Cert NatSci (Open)
Study Director (original)
Aquatic Ecotoxicology and Biodegradation

Seamus A Taylor BSc
Study Manager
Aquatic Ecotoxicology and Biodegradation

Robert A Dickinson BSc
Study Manager
Aquatic Ecotoxicology and Biodegradation (Carbon Analysis)

Georgina L Podd
Laboratory Technician
Aquatic Ecotoxicology and Biodegradation (Carbon Analysis)

Summary

The effect of (tested as a WAF; water accommodated fraction) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* was assessed under non-axenic conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 3 "Algal Inhibition Test" and the OECD Guideline for Testing of Chemicals No. 201 "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (2006).

Six replicate algal cultures, with an initial cell density of 1×10^4 /ml, were exposed to a WAF prepared from an aqueous mixture with an initial nominal concentration of 10 g/l. The test media was prepared in OECD medium by the direct addition of the test substance to the dilution medium. To aid dissolution, the test substance was stirred overnight in the dark before being left to stand for approximately 3 hours. The aqueous phase (WAF, approximately 700 ml) was then removed and used as the test medium. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 22.0 to 24.7°C for 72 hours.

The composition of the test substance made it unsuitable for analysis using chromatographic methods. Consequently, the exposure level was monitored using Total and Dissolved Organic Carbon (TOC and DOC). At the start of the test, the mean measured levels of TOC in samples of the control and test medium were 1.65 and 1.95 mg C/l respectively. The presence of algal cells made it impossible to determine TOC levels in expired test media.

Mean measured DOC levels in control and test medium were 0.55 and 0.05 mg C/l respectively at the start of the test. After 72 hours, the mean measured levels were 2.05 and 0.68 mg C/l respectively. Results of a water extractivity test conducted using a pH 7 buffered solution of at a loading rate of 10 g/l indicated a carbon content of 0.18 mg C/l. The results achieved in the test confirm the low solubility of the test substance.

Cell numbers were counted daily to monitor growth. The test results are expressed in terms of the area under the growth curve and growth rate. Compared to the control culture, neither the area under the growth curve nor the average specific growth rate was significantly reduced at a nominal loading rate of 10 g/l.

The following values were derived from the data:

Area under the growth curve

$E_b L_{50}$ (72 h) : > 10 g/l* (no inhibition)

Average specific growth rate

$E_r L_{50}$ (0 - 72 h) : > 10 g/l* (no inhibition)

No observed effect loading rate (NOELR) : 10 g/l*

* : nominal concentration of the initial aqueous mixture used to prepare the WAF.

1. Introduction

This study was designed to assess the effect of _____ on the growth of the unicellular green alga *Pseudokirchneriella subcapitata*.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 3 "Algal Inhibition Test" and the OECD Guideline for Testing of Chemicals No. 201 "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (2006). The design of the study was in accordance with recommendations received from the UK Environment Agency (May 2007), which proposed the use of a WAF.

The protocol was approved by Huntingdon Life Sciences Management and the Study Director on 7 March 2008 and by the Sponsor on 23 January 2008.

The experimental start and completion dates of the study were 24 March and 2 April 2008, respectively.

The study was conducted at Huntingdon Life Sciences Ltd., Eye Research Centre, Eye, Suffolk, IP23 7PX, England.

The composition of the test material made it unsuitable for analysis via chromatographic methods. Consequently, Total and Dissolved Organic Carbon analysis was undertaken on samples of media taken at the start and end of the test.

Information provided by the Sponsor indicated that _____ was insoluble in water. Results of a water extractability test conducted using a pH 7 buffered solution of SP polymer at a loading rate of 10 g/l indicated a carbon content of 0.18 mg C/l.

In accordance with the recommendation of the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (Number 23), the test results have been expressed in terms of the nominal loading rate (i.e. the nominal weight of the test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was removed).

2. Test substance

Identity:

Chemical name:

Lot number:

Expiry Date: 12 December 2008

Appearance: White powder

Storage conditions: Room temperature in the dark, desiccated

Purity/assay: 99.55%

Water solubility: Insoluble

Sample received (Huntingdon Site): 10 January 2008

The Sponsor's Certificate of analysis is given in Appendix 1

3. Experimental procedure

3.1 Test species

3.1.1 Name

Pseudokirchneriella subcapitata, Strain No. CCAP 278/4.

3.1.2 Source

Axenic, uni-cellular, liquid slope cultures of algae were obtained from the Culture Collection of Algae and Protozoa (CCAP), SAMS Research Services Ltd., Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, Scotland and arrived on 4 March 2008.

3.1.3 Pre-culture

The liquid slope cultures were stored in an illuminated refrigerator. Sterile algal nutrient medium (Appendix 2) was inoculated with cells aseptically removed from the slope culture; these primary liquid cultures (100 ml) were incubated for approximately three days in an orbital incubator under continuous illumination at nominal temperatures in the range 21 to 25°C. Subsequently, appropriate volumes of these primary cultures were aseptically transferred to fresh sterile algal nutrient medium to prepare secondary liquid cultures; these cultures were incubated, as stated above, for a further three days to provide an inoculum in the log phase of growth, characterised by a cell density of 1.44×10^6 cells/ml.

3.2 Culture medium

Sterile algal nutrient medium as recommended in Official Journal No. L383A Part C.3 and OECD Procedure 201 (see Appendix 2).

3.3 Test substance preparation

The method of preparation used during the test was based on recommendations by the UK Environment Agency Chemicals Assessment Unit (May 2007).

The test substance (10 g) was dispersed in culture medium (1 l) in a glass Duran bottle. This aqueous mixture was stirred overnight in the dark before being left to stand for approximately 3 hours. An aliquot (700 ml) of the aqueous phase (WAF) was then removed from mid position from the preparation vessel and was used as the test medium.

An aliquot (4.17 ml) of the secondary algal inoculum was added to a portion (600 ml) of the test medium to give an initial cell density of 1×10^4 cells/ml. An aliquot (100 ml) of the appropriate inoculated test medium was added to each of the test vessels.

3.4 Exposure conditions

3.4.1 Experimental design

The study comprised a definitive (limit) test, which employed a single test concentration, plus an algal nutrient medium control.

Six flasks were established and incubated for the control group and test group. The media remaining in the preparation flasks were used for water quality measurements and carbon analysis at the start.

Before the start of the test, the required number of empty test vessels (250 ml conical flasks), were loosely stoppered with foam bungs, covered with aluminium foil that was secured by autoclave tape and sterilised by autoclaving (121°C for at least 15 minutes). After the addition of the inoculated test medium (100 ml), each flask was then loosely plugged with a foam bung.

The control cultures were prepared as for the test medium except that no test substance was added and a larger volume (700 ml) of medium was made.

3.4.2 Test concentrations

The definitive (limit) test employed a nominal loading rate of 10 g/l. This concentration was based on the nominal weight of test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was prepared.

3.4.3 Stability of test concentrations

Total and Dissolved Organic Carbon (TOC and DOC) analysis (Appendix 3) was undertaken on samples of media taken at the start and, due to the presence of algal cells, analysis for Dissolved Organic Carbon at the end of the test.

At the start of the test, eight samples (50 ml) were taken from the freshly-prepared control media, and four from the test media; after 72 hours, the contents of the replicate flasks for each group were pooled and eight further samples were taken for analysis. All samples were stored in a freezer until defrosted for analysis when required.

Two samples from the start of the test were analysed for Total Organic Carbon and two were filtered (0.45µm cellulose nitrate) before analysis for Dissolved Organic Carbon. At the end of the test, four samples were analysed for Dissolve Organic Carbon.

3.4.4 Environmental conditions

Conical flasks (250 ml) each containing control or test culture (100 ml) were placed in an illuminated orbital incubator according to a random number sequence. The cultures were incubated, without renewal of medium for 72 hours under continuous illumination of approximately 6499 lux provided by 6 x 30 W "cool white" 1 metre fluorescent tubes. The temperature was maintained at 22.0 to 24.7°C (see protocol deviations).

Temperature and pH of control and test flasks at the start and end of the test were recorded. Gaseous exchange and suspension of the algal cells were ensured by the action of the orbital shaker, oscillating at a nominal 150 cycles per minute. The minimum and maximum temperature and light intensity in five positions within the test area (four corner positions and in a central position of the random block design) were determined each day. To minimise the impact of differences in light intensity across the test area on algal growth, control and test flasks were re-positioned in the test area each day during the test.

3.5 Measurement of growth

Samples were taken from control and test flasks at 24, 48 and 72 hours and the cell densities measured using a Coulter Z Series Particle Count and Size Analyser.

The estimate of cell numbers in each sample was based on the mean of three consecutive counts, corrected for background counts of uninoculated dilution media. The presence of any abnormal cells was also noted during screening of each test level.

3.6 Evaluation of data

The data were compiled in an Excel spreadsheet and analysed using SAS 8.2 (SAS Institute 1999) using the nominal loading rate.

The areas under the growth curve were divided by initial counts and total times to give AUCP (Area Under the Curve expressed as a Proportion of the initial cell count), where a value of 1 represents no growth and a value of 0 represents complete toxicity (all algae killed). In order to estimate the loading rate at which 50% inhibition of growth occurred (EL_{50}), sigmoidal curves were fitted to AUCP and growth rate. For both variables, 0% inhibition was defined as the control mean and 100% inhibition was defined as no growth. The minimum of the curve (for infinite concentration) was bounded between 0 and 1 for AUCP and between -1000 and 0 for growth rate.

The formulae for these curves are given below:

$$AUCP = \begin{cases} \frac{Con}{Min} + \frac{Con - Min}{50(Con - 1)ep} & \text{Control} \\ 1 + \frac{50(Con + 1) - 100Min}{50(Con - 1)ep} & \text{Otherwise} \end{cases} \text{ where } ep = \exp\left(s \log\left(\frac{\text{concentration}}{EC50}\right)\right)$$

$$\text{Growth rate} = \begin{cases} \frac{Con}{Min} + \frac{Con - Min}{50Con ep} & \text{Control} \\ 1 + \frac{50Con - 100Min}{50Con ep} & \text{Otherwise} \end{cases}$$

Con = an estimate of the control mean

Min = an estimate of the minimum of the curve

s = slope estimate

The EL_{10} and EL_{50} values could not be calculated because no inhibition of growth was noted.

For AUC and growth rate, the *t*-test was used to compare the treated group with the control.

3.7 Protocol Deviations

During the test, the temperature of the incubator ranged between 22.0 and 24.7°C, which deviated from the range stated in the protocol (21 to 24°C).

This had no impact on either the integrity or validity of the study as all validity criteria were met.

4. Maintenance of records

All specimens (if appropriate), raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Archive.

Specimens and records will be retained for a minimum period of one year from the date of issue of the final report. At the end of the one year retention period the Sponsor will be contacted and advice sought on their future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

5. Results

5.1 Chemical analysis

The results of the carbon analysis are given in Table 1.

At the start of the test, the mean measured (blank corrected) levels of TOC in samples of the control and test medium were 1.65 and 1.95 mg C/l respectively. The presence of algal cells prevented TOC analysis in expired test media.

Mean measured (blank corrected) DOC levels in control and test medium were 0.55 and 0.05 mg C/l respectively at the start of the test. After 72 hours, the mean measured levels were 2.05 and 0.68 mg C/l respectively.

In accordance with the recommendation of the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (Number 23), the test results have been expressed in terms of the nominal loading rate (i.e. the nominal weight of the test substance used to prepare the aqueous mixture from which the WAF (water accommodated fraction) was removed).

5.2 Algal growth

Cell numbers were counted daily to monitor growth. The test results are expressed in terms of the area under the growth curve and growth rate.

Compared to the control cultures, neither the area under the growth curve nor the average specific growth rate was significantly reduced at a nominal loading rate of 10 g/l. Based on these findings, the following values were derived from the data:

Area under the growth curve E_bL_{50} (72 h) : > 10 g/l* (no inhibition)

Average specific growth rate E_rL_{50} (0 - 72 h) : > 10 g/l* (no inhibition)

No observed effect loading rate (NOELR) : 10 g/l*

* : nominal concentration of the initial aqueous mixture used to prepare the WAF.

The mean coefficient of variation (CoV) for daily growth rates in control cultures ranged between 3.36 and 4.27 during the test and the CoV for the average specific growth rates of control cultures was 1.17 during the 72 hour exposure period.

5.2.1 Observations

No microscopic abnormalities of the cells were detected.

5.2.2 Environmental parameters

The measurements of water quality (temperature and pH) in control and test flasks are summarised in Table 4; they remained within acceptable limits throughout the study.

The temperature of the incubator ranged between 22.0 and 24.7°C (see protocol deviation). Measurement of light intensity ranged between 6424 and 6584 lux (mean values) during the test and were within the range -5.4 and +7.7% (Table 4).

At the start of the test, the test medium was colourless.

6. Conclusions

Under the conditions of the study, _____ was not found to be acutely toxic to *Pseudokirchneriella subcapitata* when tested as a WAF at a nominal loading rate of 10 g/l.

Consequently, the 72-hour E_bL_{50} and E_rL_{50} values for _____ could not be calculated but must be >10 g/l and the "no observed effect loading rate" was 10 g/l.

7. References

OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (Number 23) ENV/JM/MONO(2000)6

SAS INSTITUTE (1999) SAS OnlineDoc® *Version Eight*. SAS Institute Inc., Cary, NC, USA.

UK ENVIRONMENT AGENCY CHEMICALS ASSESSMENT UNIT
RECOMMENDATIONS (2007). Notification of New Substances Regs. (1993): Level 1B
Testing Proposal

Table 1 Measured TOC and DOC concentrations

| Nominal Loading Rate (g/l) | Measured TOC concentrations (mg C/l) | |
|-------------------------------|--------------------------------------|------|
| | 0 hours | |
| Control | 0.3 | 3.0 |
| | (1.65) | |
| 10* | 2.25 | 1.65 |
| | (1.95) | |

* : blank corrected values (measured concentration - mean control value for sampling occasion)

() : mean measured concentration

| Nominal Loading Rate (g/l) | Measured DOC concentrations (mg C/l) | | | |
|----------------------------------|--------------------------------------|------|----------|------|
| | 0 hours | | 72 hours | |
| Control | 0.5 | 0.6 | 2.0 | 2.2 |
| | (0.55) | | | |
| | - | - | 2.1 | 1.9 |
| | - | | (2.05) | |
| 10* | 0.05 | 0.05 | 2.65 | 0.05 |
| | (0.05) | | | |
| | - | - | 0.0 | 0.0 |
| | - | | (0.68) | |

* : blank corrected values (measured concentration - mean control value for sampling occasion)

() : mean measured concentration

Table 2 Cell densities

| Nominal Loading Rate (g/l) | Replicate number | Cell densities ($\times 10^4$ cells/ml) | | |
|----------------------------|------------------|--|----------|----------|
| | | 24 hours | 48 hours | 72 hours |
| Control | R ₁ | 46317 | 265750 | 1189017 |
| | R ₂ | 50450 | 265283 | 1320450 |
| | R ₃ | 51550 | 256283 | 1264583 |
| | R ₄ | 48383 | 226017 | 1123150 |
| | R ₅ | 43583 | 234750 | 1239817 |
| | R ₆ | 48450 | 256650 | 1262417 |
| | Mean | 48122 | 250789 | 1233239 |
| 10 | R ₁ | 53683 | 245517 | 1073850 |
| | R ₂ | 55983 | 258350 | 1139250 |
| | R ₃ | 54517 | 238983 | 1212517 |
| | R ₄ | 57017 | 239117 | 1229550 |
| | R ₅ | 58383 | 265850 | 1254450 |
| | R ₆ | 55317 | 251117 | 1227550 |
| | Mean | 55817 | 249822 | 1189528 |

R₁-R₆ : replicate number.

Note : the initial cell density was estimated to be 1.03×10^4 /ml.

Table 3 Inhibition of growth

| Parameter | Nominal Loading Rate | Sample size | Mean | % Inhibition | <i>p</i> |
|------------------------------|----------------------|-------------|-------|--------------|-----------|
| Area under curve to 72 hours | Control | 6 | 21.4 | 0.0 | - |
| | 10 g/l | 6 | 21.0 | 1.7 | 0.560 |
| Growth rate to 72 hours | Control | 6 | 0.066 | 0.0 | - |
| | 10 g/l | 6 | 0.066 | 0.8 | 0.304 |
| Growth Rate 0 - 24 hours | Control | 6 | 0.065 | 0.0 | - |
| | 10 g/l | 6 | 0.071 | -9.5 | <0.001*** |
| Growth Rate 24 - 48 hours | Control | 6 | 0.068 | 0.0 | - |
| | 10 g/l | 6 | 0.062 | 9.2 | <0.001*** |
| Growth Rate 48 - 72 hours | Control | 6 | 0.066 | 0.0 | - |
| | 10 g/l | 6 | 0.065 | 2.1 | 0.370 |

p values are for the comparison with Control using the *t*-test

*** *p* < 0.001

Table 4 Environmental parameters**a) Temperature and pH**

| Nominal Loading Rate (g/l) | Temperature °C | | pH | |
|-------------------------------|----------------|------|------|------|
| | 0 h | 72 h | 0 h | 72 h |
| Control | 22.4 | 22.1 | 7.61 | 8.27 |
| 10 | 22.3 | 22.1 | 7.69 | 8.36 |

b) Light intensity

| Incubator Position | Exposure (days) | | | |
|-----------------------|--------------------|--------------------|--------------------|--------------------|
| | 0 | 1 | 2 | 3 |
| | Lux | | | |
| Top/left | 6840 | 6370 | 6460 | 6430 |
| Bottom/left | 6430 | 6330 | 6420 | 6430 |
| Centre | 6970 | 6920 | 6990 | 6780 |
| Top/right | 6230 | 6240 | 6310 | 6440 |
| Bottom/right | 6450 | 6260 | 6370 | 6310 |
| | | | | |
| <i>mean</i> | <i>6584</i> | <i>6424</i> | <i>6510</i> | <i>6478</i> |
| <i>% variation</i> | <i>-5.4 / +5.9</i> | <i>-2.9 / +7.7</i> | <i>-3.1 / +7.4</i> | <i>-2.6 / +4.7</i> |

Appendix 1 Certificate of Analysis**CERTIFICATE OF ANALYSIS**

NAME OF SAMPLE

LOT NO. OF SAMPLE :

DATE OF ANALYSIS : 12 December 2008

COMPOSITION

| Identity | Cas No. | Conc. |
|--------------|---------|-------------|
| #4 | | % |
| #5 | | % |
| TOTAL | | 100% |

NAME

SIGNATUREDATE 23 June 2008

Appendix 2 Algal Nutrient Medium (OECD)

Four stock solutions were prepared according to the following table, using filtered, dechlorinated tap water which had been softened and treated by reverse osmosis, before microfiltration and purification (resistivity of 18 Megohm/cm). Stock solutions were sterilised by autoclaving (solutions 1-3) or by membrane filtration (solution 4) before being stored at 4°C in the dark.

Aliquots of stock solutions 1-3 were further diluted with the same diluent and autoclaved again to produce the working strength nutrient medium. Stock solution 4 was added to the medium on the day of use. The pH of the medium after equilibration with air is approximately 8.

| Nutrient | Concentration in stock solution (g/l) | Volume of stock solution per litre of final medium (ml) | Final concentration in test solution (mg/l) |
|---|---|--|--|
| Stock solution 1: macro-nutrients | | | |
| NH ₄ Cl | 1.5 | 10 | 15 |
| MgCl ₂ .6H ₂ O | 1.2 | | 12 |
| CaCl ₂ .2H ₂ O | 1.8 | | 18 |
| MgSO ₄ .7H ₂ O | 1.5 | | 15 |
| KH ₂ PO ₄ | 0.16 | | 1.6 |
| Stock solution 2: Fe-EDTA | | | |
| FeCl ₃ .6H ₂ O | 0.064 | 1 | 0.064 |
| Na ₂ EDTA.2H ₂ O | 0.1 | | 0.1 |
| Stock solution 3: trace elements | | | |
| H ₃ BO ₃ | 0.185 | 1 | 0.185 |
| MnCl ₂ .4H ₂ O | 0.415 | | 0.415 |
| ZnCl ₂ | 3 x 10 ⁻³ | | 3 x 10 ⁻³ |
| CoCl ₂ .6H ₂ O | 1.5 x 10 ⁻³ | | 1.5 x 10 ⁻³ |
| CuCl ₂ .2H ₂ O | 10 ⁻⁵ | | 10 ⁻⁵ |
| Na ₂ MoO ₄ .2H ₂ O | 7 x 10 ⁻³ | | 7 x 10 ⁻³ |
| Stock solution 4: NaHCO₃ | | | |
| NaHCO ₃ | 50 | 1 | 50 |

Appendix 3 Total and Dissolved Organic Carbon Analysis (TOC and DOC)

Introduction

TOC or DOC concentrations in aqueous mixtures were determined using an OI Model 700 carbon analyser. Samples for DOC analysis were prepared by filtration of the medium (0.45 µm membrane filters).

Organic carbon concentrations were measured in this analysis by the amount of carbon dioxide released by chemical oxidation of the aqueous sample at 100°C in the presence of phosphoric acid and sodium persulphate. The carbon dioxide was purged from the sample, concentrated by trapping and desorbed and carried to a non-dispersive infrared detector (NDIR) whose output was calibrated (using aqueous solutions of potassium hydrogen phthalate) to directly display the concentration of carbon present in the sample. Sample volumes were measured using a calibrated sample loop (1.02 ml) and phosphoric acid then sodium persulphate were automatically injected with each sample.

Materials

Chemicals used in carbon analysis

Potassium hydrogen phthalate (Analar grade) used as a standard.

Sodium persulphate (98+%).

Phosphoric acid (85% Orthophosphoric acid, analytical grade; SG 1.7) for acidification of samples as part of the analysis in "TIC/TOC" mode.

Dilution water

The dilution water used to prepare solutions was tap water that had been softened and treated by reverse osmosis then purified; resistivity $\geq 18 \text{ M}\Omega\cdot\text{cm}$. This water complied with the relevant British Standard and American Society for Testing and Materials for classification as Grade 1 or Type 1 Laboratory Water, respectively.

Determination of carbon levels

The carbon analyser was calibrated using at least five samples of an aqueous solution of potassium hydrogen phthalate (20 mg C/l). At least five samples of ultrapure water verified the absence of contamination within the carbon analyser and the TOC content of five samples of the reagents (sodium persulphate and phosphoric acid) were analysed to obtain a mean blank value in the calibration. These reagent blanks were entered into the calculation program of the analyser. The potassium hydrogen phthalate solution was analysed at the end of each set of analyses and verified the stability of the detector response.

The TOC/DOC content of dilution medium control, solvent control and test medium was determined in the TIC/TOC mode of analysis. Samples injected into the analyser were acidified (phosphoric acid; 0.4 ml of a 5% v/v solution) then purged with nitrogen in order to release inorganic carbon. The oxidant (sodium persulphate; 1.0 ml of a 100 g/l solution) was automatically added to the sample and the mixture heated to approximately 100°C in the analyser to oxidise the organic carbon to carbon dioxide which was quantified.

Appendix 4 Eye Research Centre GLP Compliance Statement 2008



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

28th January 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

4/03/08

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

